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TRIAL OF AN AVIAN INFLUENZA SURVEILLANCE SCHEME IN THE MOEYINGYI WETLAND OF MYANMAR

Direttore della scuola: Ch.mo prof. Gianfranco Gabai

Coordinatore di indirizzo: Ch.mo prof. Mauro Dacasto

Supervisore: Ch.mo prof. Marco Martini

Dottorando: Alessandro Cristalli

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2. SYNTHESIS

2.1 Introduction

Avian Influenza (AI) is a transmittable disease of birds caused by a Type A Orthomyxovirus. Infected birds can show different clinical patterns according to the species and to the virus pathogenicity. The virus can spread easily from bird to bird at poultry markets, through commercial routes such as poultry markets and also through the migration of wild birds and is a typical transboundary disease (Alexander, 2009). AI can have serious socio-economic and public health consequences. Among the Avian Influenza Viruses (AIV), the Highly Pathogenic Avian Influenza (HPAI) virus H5N1 subtype is the most relevant one circulated in South East (SE) Asia in the last decade, causing regional and global epidemics. It first emerged in SE Asia at the end of 2003 and spread westward into Europe despite preventive measures that were put in place.

Small scale family poultry farming is an important livelihood source for the poor in the tropics. Tropical household poultry farming is usually based on rearing local varieties of the *gallus gallus* species of the various species of the *anatidae* family. These indigenous varieties are often not recognized as breeds due to a lack of systematic description. Family poultry rearing requires neither access to land nor even land use, and is a typical livelihood activity for landless poor. It is a typical low external input practice based on simple technology and knowledge.

In developing countries and in particular in SE Asia, household poultry rearing has a great importance for poverty reduction. In addition, it is typically carried out by women who can look after all the productive stages from hatching to sale and directly earn money. The poultry sector of Myanmar can be broken down into a formal sector and a traditional one. The first is market oriented and is differentiated into different subsectors with different levels of reliance on technology. The first H5N1 HPAI outbreak reported and notified in Myanmar was in March 2006. Three distinct HPAI epidemic waves followed and involved 6 States/Divisions. Post outbreak surveillance was put in place but was withdrawn afterwards and did not contribute to the establishment of a real early warning system. Between 2007 and 2008 two other epidemics spread and were controlled. As of July 2011, the last notified outbreak was in 2011 (OIE, 2011).

This research contributed to increasing the knowledge on the influenza viruses circulating in Myanmar with a specific investigation on the Moeyingyi wetland and made information and knowledge available to the national veterinary services.

The present doctoral research was managed by the doctoral researcher in collaboration with the Istituto Zooprofilattico Sperimentale Delle Venezie (IZSVe) and the Livestock Breeding and Veterinary

Department (LBVD) of Myanmar, which is the managing structure of the veterinary services of the Union of Myanmar.

2.2 Materials and Methods

The research project was comprised of 4 phases with different objectives. Each phase followed a determined methodology. The first three phases used tools closer to development disciplines rather than formal science, while the fourth was focused on field epidemiology and followed a strict protocol. Project Phases 1, 2 and 3 were preparatory to the implementation of the epidemiological study and at the same time produced necessary information.

Project **Phase 1** envisaged a survey carried out by means of a Rapid Rural Appraisal (RRA) carried out in February 2008.

Project **Phase 2** envisaged gathering secondary information to be validated through a participatory workshop designed for the exchange of information among stakeholders and for information triangulation (April 2008).

Project **Phase 3** entailed collecting, organizing, and managing new data through the implementation of an agro-ecosystem analysis (AEA) started in April 2008 with the purpose of identifying and describing duck farming practices and their integration with the rice farming cycle, with attention to seasonal trends, climate changes and other ecological aspects such as the watershed water flow pattern. The AEA approach allowed for the identification of four strata with roughly homogeneous environmental and farming characteristics. These strata coincided with those of the subsequently developed stratified randomized sampling plan.

Based on the information gathered, a questionnaire to rationalize the collection and recording of risk factors was developed. The questionnaire "zero draft" was discussed during the workshop carried out in April 2008.

Once the questionnaire was finalized, an electronic database was developed to enable the systematic registration of data collected.

After these three phases were accomplished, Project **Phase 4**, the epidemiological study entitled "Trialing of an Avian Influenza Surveillance System in the Moeyingyi Wetland of Myanmar," was started. This study is the core of the Doctoral research. The implementation of the epidemiological study was parallel to the implementation of a social-economical investigation carried out by the FAO in association with the NGO Winrock International. The objective was to obtain epidemiologic scientific information and a social-economical insight into the current situation in order to enable the development of control measures sustainable for local conditions.

2.2.1. Protocol of the epidemiological study

The epidemiological study has a longitudinal design which envisaged:

- one initial cross sectional sampling addressing 40 duck farms (units) out of a sampling frame of 1.769 units. A random stratified balanced sample was used.

- The resulting negative units constituted the “negative cohort” for further follow up.

- Units which turned positive exited the study.

- 5 sampling rounds were carried out to sample the negative cohort. Sampling rounds were carried out along the entire duration of a production cycle and across different seasons (8 months).

- When the negative cohort became small new negative units were added. The negative units were identified by means of a second cross sectional study carried out on 40 newly randomly selected farms.

- Intervals between sampling round were variable between 30 and 60 days. The sampling moment was set according to the occurrence of relevant moments of the rural life.

- In those cases where the randomized selection of units was fulfilled the systematic sampling was adopted.

- The 4 identified strata correspond to the agro-ecological zones identified during the AEA. They are

  Stratum 1: West side of the Moeyingyi Lake or “roadside,”

  Stratum 2: Moeyingyi Lake, or “lake,”

  Stratum 3: Southern side of the lake or “bank,”

  Stratum 4: Bago-Sittaung canal, or “canal.”

2.2.2.1 Sampling protocol

In each unit the operations were standard and envisaged:

- Registration of GPS coordinates.

- Collection of at least 30 blood samples from randomly selected ducks

- Collection of at least 30 specimens for virological analysis. Tracheal or oropharyngeal swabs (according to the availability of specific swabs) from randomly selected ducks

- Administration of one questionnaire for registering risk factors’ occurrence (previously identified and defined).

In determined units where serum-conversion was identified to have occurred within the previous two weeks, intensive sampling covering between 80-100% of subjects was envisaged in order to facilitate the isolation of freshly shed virus.

2.2.2.2. Epidemiological analysis

The epidemiological analysis entails

- A **descriptive** analysis outlining the sero-prevalence and incidence per stratum and with details of the farm level.

- A **survival analysis** to evaluate the serum-conversion time for the farm investigated

2.3 Results

Only results generated by Phase 3 (AEA) and Phase 4 (epidemiological study) are reported. These results are more strictly related to the objectives of this doctoral thesis and have been worked out by means of the application of consistent observations (phase 3) and scientific investigation methods (Phase 4).

2.3.1 Results of the agro-ecosystem analysis (AEA) – Project Phase 3

The area investigated is generally called *Moeyingyi Wetland* and is located in the Bago East Division. It is divided into four townships whose veterinary authorities have been active in conducting the field survey. The area is characterized by the presence of the Moeyingyi Lake which has an average surface area of 16 hectares and is surrounded by a swampy land submerged or dry according to seasonal variability. The area is registered as a RAMSAR protected area since 2004 and hosts 125 species of migratory and residential wild birds.

Intriguingly, it is also the biggest reproductive hub of domestic ducks in Myanmar. Traditional methods are used to supply eggs and ducklings to most of the country.

The portion of land south of the earth bund lake edge is crossed by an artificial canal called the “Bago-Sittaung canal” which was dug across the Waw and Thanatpin township territories by the English colonizers. It is 60 Km long and connects the Sittaung and Bago rivers.

The area is characterized by secondary and tertiary canals which feed the Bago-Sittaung canal. Their surrounding is classified as rice cultivated land whose flooding is governed through gates which release the water outflow from the lake through the artificial earth bund into a network of irrigation canals which deliver water to the fields. The population of domestic waterfowl was estimated with the best possible accuracy considering seasonal variation to range between 2.000.000 and 4.000.000 subjects. It is grouped into 100 – 4000 subject flocks housed across the four agro-ecological zones.

Ducks are confined during night-time and are conducted to water bodies in day-time. The grazing diet is supplemented by shrimps, broken rice and shells. Reproduction is centralised in 12 traditional hatcheries connected with occasional breeding farms.The local rearing practice requires low external input but also has low biosecurity. Ducks are housed in pillar shelters made of bamboo. The internal area is barely separated into two areas: one houses ducks and one is for the farming family. The separation is provided by a net which maintains a weak bamboo fence. The roof is built on a bamboo frame supporting patches of palm leaves and rice straw. The practical and ingenious engineering of the houses combined with building simplicity and the lightness of bamboo is functional to disassembling and rebuilding allowing for easy relocations. Flocks make seasonal movements following expansion and retraction of the lake shore and the flooding of rice paddies.

Rice cultivation and the duck rearing cycle are integrated and both depend on water availability. Ducks graze on flooded paddies feeding on vegetables, algae, mollusks and insects, or wander in dried harvested rice fields picking up leftover grains. This brings mutual benefits to both the land owner and the duck farmer. It is worth reporting that several observations highlight that the local duck breed has a strong flock instinct and has a body conformation well suited to rapid movement on dry land. Thanks to these characteristics the flock is shepherded around and driven to rice paddies by means of exclusively vocal and visual orders. The ducks are small in size, reaching 1.5 – 2 kg of live weight. The productive cycle usually starts with the purchase of 3-9 day old ducklings and lasts, theoretically, until ducks are spent and are finished to be sold for meat purposes. In reality, observations carried out along the time dimension in the Project Phase 4 show that the production cycle is rarely completed due to many externalities which determine the sale of the flock. The business is intermittently lively due to the fluctuation of the egg price. At the time of the AEA the egg price was equal to 85 Kyat (€ 0.06) but price fluctuations were reported to be very wide, undermining any business forecast and postponing the foreseen return on investment.

2.3.2 Results of the epidemiological study – Project Phase 4.

16 out of 80 duck farms stepped out of the study due to causes not related to AI. The largest defection occurred in the two last sampling rounds. Results are summarized by sampling round as follows:

First Sampling (September 2009):

- 22 /40 duck farms positive for Ab against AIV subtype H5.

  Prevalence rate 55%.

- 18 / 40 duck farms negative.

- Virus isolation to the embryonated eggs was negative.

Second sampling (October 2009):

- 4/18 duck farms positive for Ab against AIV subtype H5.

  Incidence rate 22% .

- Virus isolation negative.

Third sampling (November 2009):

- 2/13 duck farms positive for Ab against AIV subtype H5. Incidence rate 15%.

- 22 out of 40 newly introduced duck farms were positive for Ab against AIV subtype H5 with a prevalence rate of 55%.

- 5 duck farms left the business and were excluded from the study.

Fourth sampling (January 2010):

- 5/21 duck farms positive for Ab against AIV subtype H5. Incidence rate 24%.

- 8 duck farms left the business and were excluded from the study.

Fifth sampling (March 2010):

- 3/9 duck farms positive to Ab against AIV subtype H5. Incidence rate 67%.

The survival analysis was possible on 20 out of the 80 farms investigated. It showed that one month after the first sampling, the probability of not being infected was 93% while at 3 months the probability was 65%. After 4 months it fell to 38% and by the end of the study it was close to 0. The median time for seroconversion was 4.23 months.

2.3.3 Discussion and conclusions

The Moeyingyi wetland is a complex agro-ecosystem originating from an ecosystem set under human control by means of the construction of the Bago-Sittaung canal during the colonial era and of the recent water gate governed network of irrigation canals which detaches from the earth bund at the lake's southern shore. These interventions permitted the settlement of farmers who make their living on rice cultivation integrated with duck farming. The core of the original ecosystem is preserved in the lake itself as a wild bird sanctuary but is intermingled with the surrounding agro-ecosystem. Domestic ducks often mix up with wild birds, creating favorable conditions for the transmission of AIV between the two populations. The epidemiological study demonstrated that the suspicion was confirmed and that there is a high rate of H5 AIV subtype circulation. Intriguingly, despite a high incidence of infection (new serum positive flocks) and although the newly serum-converted farms were timely sampled for virological investigation, virus was never directly detected, nor the pathotype identified. Speculations about the reasons for justifying the unexpectedly high serological titres (confirmed by the Italian NRL) led the researcher to hypothesize that they were induced by a Highly Pathogenic Avian Influenza Virus or that, alternatively, they were boosted to high levels by repeated natural infections with Low Pathogenic Avian Influenza Viruses.

In terms of research achievements and in order to facilitate easier evidence based decision making, virus isolation remains necessary. In the local socio-economic conditions, the absence of symptoms in domestic waterfowl and the absence of the isolation of virus do not enable decision makers (veterinary services) to easily take action and implement unpopular and expensive control measures.

Although the study provided a serological explanation of virus circulation, laboratory testing for virus isolation and RT-PCR did not produce results consistent with the epidemiological scenario. Even if the field staff was in place in time to sample some farms at the suspected time of viral shedding, the lack of identification of H5 genes reflects the complicating role of the unpredictable, short, and intermittent shedding after the infection.

Serological results have enabled the execution of a survival analysis that identified and quantified the time of the spread of the infection starting from the housing of ducklings (August- September) until the end of the production cycle (May).

Main findings of the Survival Analysis

- 2 months after the first sampling, farms of the roadside stratum had lower probability to get infected (around 54%) in comparison to the strata canal, bank and lake (probability above 75%)

- After 3 months, duck farms of the stratum canal continue to be less susceptible to the infection (probability 76%) while the two strata bank + lake show a trend comparable to the stratum roadside

- After 4 months duck farms located in the stratum LAKE SIDE + LAKE have a stable probability of being infected (30%) while in the other strata some farms remained uninfected after 6 months

Bearing in mind these research results, a high intensity of surveillance in the area is recommended. The surveillance scheme carried out needs be improved in order to increase the possibility of viral isolation, while using human resources and funds in a cost/effective way. Even though the presence of an OIE notifiable subtype of AIV was detected indirectly, the impossibility of its isolation complicates the issue. It is therefore suggested to plan, execute, monitor and evaluate a detailed virological surveillance plan based on the suggestions originated from the present study.

It is recommended to pursue the development and implementation of control measures which are sustainable. This can be achieved if local development is pursued at the wider level and a long term strategy fostering economic changes by reducing duck farming and planning alternative livelihood sources is put in place.

2.4 Summary

Myanmar was infected for the first time by the H5N1 Highly Pathogenic Avian Influenza virus subtype in March 2006. Two other epidemics occurred in 2007 and 2008, and after two years of no report, at the beginning of 2010 two epidemics occurred, and another at the beginning of 2011. They were controlled by means of stamping out.

In the country, systematic surveillance for AI is not yet an established practice. Cross sectional studies are currently being started in some risk areas.

The present research tests a surveillance scheme for HPAI by means of a longitudinal design and was carried out in the Moeyingyi wetland and its wild bird sanctuary (Bago East District). The Moeyingyi area hosts 125 avian species and approximately 3000 semi-commercial duck farms for a total of 2-4 million native laying ducks and produces around the 60% of the ducklings of Myanmar.

Physically, the Moeyingyi Lake is located in the central of the studied area and has undefined shores connected with a network of irrigation channels.

Farms, either resident or making short transhumances, rear ducks up to two laying cycles. Ducks are confined during night-time and are conducted to water bodies in day-time. The grazing diet is supplemented with shrimps, broken rice and shells. Reproduction is centralised in 12 traditional hatcheries connected with occasional breeding farms.

Descriptive epidemiology was carried out. A survival analysis was performed on 20 of the 80 farms involved in the study. Due to the impossibility to isolate AIV and the abundance of Ab data, the results stem from serological testing. We hypothesized that the virus isolation was negative given the short viral shedding time after infection. Only in 3 cases we could test farms suspected of being infected over the previous 2 weeks. Nevertheless the isolation remained negative.

Serological incidence indicates that an H5 epidemic propagates quickly and constantly during the production cycle. This is confirmed by the survival analysis which showed the increasing probability of being infected. There were no significant differences when comparing different agro-ecosystems.

In conclusion, to control AIV spread it is recommended to enforce control measures in all the agro-ecosystems whenever new flocks are housed.

3. INTRODUCTION

3.1. Problem analysis at the wider level

3.1.1. Risk of AI epidemics on the global scale originating from SE Asia.

Avian Influenza is an epidemic transmittable disease of poultry which can generate epidemics on regional and global scales. In particular the HPAI H5N1 subtype which emerged at the end of 2003 in SE Asia generated a regional crisis and impacted all the sectors of poultry farming. The epidemic spread to European countries which notified H5N1 HPAI occurrence to the OIE. Some outbreaks had secondary follow-ups while others were promptly cleared and the infection immediately eradicated. The list of the notified index cases occurred in European countries is reported below.

Romania	04/01/06
Ucraine	10/01/06
Croatia	17/01/06
Bulgaria	12/02/06
Slovenia	11/02/06
Greece	13/02/06
Italy	14/02/06
Germany	16/02/06

Austria	20/02/06	
France	20/02/06	
Hungary	20/02/06	
Slovak Republic	24/02/06	
Switzerland	26/02/06	
Check Republic	22/06/07	
Poland	03/12/07	
United Kingdom	03/02/07	(OIE, 2011).

These outbreaks occurred even as veterinary services of European Countries intensified surveillance and control measures. It is therefore arguable that until there is no virus circulating in SE Asian countries, the risk for H5N1 HPAIV reintroduction into EU poultry flocks will not be negligible. Interestingly, the FAO fostered the strategy of "Combating Avian Influenza at its source" which was a benchmark of its approach". The first point of the so called "three-pronged approach" was "working with all affected and at risk countries to strengthen veterinary services and to improve local capacity at the farm and market level" (Domenech *et al.*, 2006). This doctoral research contributes to this point and aims at reducing the risk of global AI epidemics. The present work responds to the globally identified emergency by contributing doctoral research which is also legitimized by the mentioned policy framework.

3.1.2. Socio-economic importance of the disease in SE Asian countries

3.1.2.1. Social importance of poultry farming

Poultry farming is economically relevant in tropical developing countries. The poor often engage in the small scale farming of indigenous poultry. These birds are popular because they are well adapted to the environment and to the local culture. They are characterized by slow growth and low nutritional requirements. This kind of poultry breeding does not require externally provided technology or inputs, but there is some integration with by-products of the main local cultures. This type of poultry farming is sometimes identified as “sector 4” by the FAO (FAO, 2006) and fits with the needs of landless people because their poultry are typically free-ranging on common lands or on others’ land. There is also an acknowledged correlation of small scale chicken and hen rearing with women conducted businesses. Men tend to practice more market oriented models and, in particular, are more employed in duck farming (Velasco *et al.*, 2008). We avoid speculating on the anthropological reasons, but is worth mentioning that ducks have characteristics (for instance a strong flock instinct) which favor their movements in big groups. They are therefore suitable for itinerant models of management and for this reason are mostly reared by men who usually hold the stronger social status needed to engage in negotiations with the owners of the pastureland. The researcher has observed that in many marginal areas and difficult environments duck farming often requires transhumances of different length which sometimes require the farmer to stay alone away from home. In conclusion, the need to have bargaining power *vis a vis* with other farmers in male biased cultural environments makes this type of duck farming eligible for men.

Chicken farming is mainly resident and less integrated with land use planning and cycles of cultures. It is an activity which fits for the most disadvantaged people like those with disabilities, pregnant women, elders, and children. It is worth dispelling doubts about the impact that family poultry farming has on malnutrition. Family poultry farming reduces food insecurity but own poultry is only eaten for celebrations or for traditional medicine preparations, and the majority is sold in the marked. The earnings are then spent to buy starchy staple foods such as rice or other cereals. The myth that small scale poultry farming provides proteins to the poorest must be revised and only holds for egg consumption which is mostly restricted to children during the schooling period. Free ranging family poultry farming provides the landless with the possibility of exchanging some otherwise unobtainable goods with other nutritious food. Field observation shows that in Vietnam, Cambodia, and Myanmar indigenous breeds reared with traditional methods have a market value 2,5 times greater than industrially reared poultry. Small scale family poultry farming is also acknowledged as an effective tool for the empowerment of women, who can entirely follow, manage, and control the various productive phases. They can manage the income and provide benefits for the family. Small scale traditional poultry farming also has social and anthropological values which must be considered when the poultry sector becomes the object of restructuration towards industrial models. In practical terms the eradication of poultry disease is unsustainable when approached through the eradication of traditional family poultry farming. This practice will be impossible to stop in the countryside for the above mentioned reasons. Small scale poultry family farming cannot be readily replaced because it has a great importance in peoples' livelihood and also in their social and cultural life.

3.1.2.2. Economic importance of poultry farming in SE Asian countries

Agriculture provides 50% of the Myanmar Gross Domestic Product (GDP). 10% of this share is provided by livestock farming. In the first decade of 2000, the industrial poultry sector showed a growth rate more than double those of other agricultural and other primary production activities (Burgos *et al.*, 2009).

The pattern of development occurring in Myanmar follows the steps observed in neighboring countries. In the region, developing economies which mainly rely on agriculture can rapidly grow GDP through export oriented agro-industrial development. The aggregate GDP growth corresponds to a welfare distribution usually skewed to the right, benefiting urban people whose purchasing power for countryside agro-products increases dramatically. This phenomenon is also economically referred to as change of the "term of trade" for urban elites (Livingstone, 1981) and ends up in an increased demand for certain foods. The Engel law (Zimmerman, 1932). explains that the offer – demand curve is not income elastic for all the food items. Some (such as staple foods) are relatively inelastic to income growth, while others (such as more expensive or special foods) are more demanded by urban people with higher incomes (Zimmerman, 1932). Meat is the most typical example of income elastic food. This is explained by Bennet's Law (Joshy *et al.*, 2007). According to observations (Gulati *et al.*, 2007), traditional poultry production responds first to demand and can supply the type of meat appreciated by urban consumers. Traditional tastes are still preferred by the newly settled urban elites whose memory and ties with the countryside are still strong. The demand also stimulates an increase in poultry production in the industrial sector. This figure is quite standard and follows well known market laws. In practice, this stimulus triggers the establishment of rural-periurban-urban poultry value chains which supply live poultry from the countryside towards cities and which consequently increases the risk of spread of avian diseases or food borne pathologies. It is worth noting that in tropical developing countries food supply chains rarely have refrigeration and, in the absence of a cold chain, transportation of live animals is preferred. Livestock and poultry are then slaughtered in places as close as possible to the location of consumers.

Moreover, for obvious logistical reasons, transportation of live animals is easier with poultry than with swine or cattle.

Investors, mainly transnational agro-industrial companies, do not hesitate to catch these business opportunities and develop profitable industrial poultry enterprises, often integrated with feed production systems.

The phase of agro-business led development is common to SE Asian countries where integrated complexes mushroom in the areas surrounding cities. Observations and interviews made by the researcher in 2007 (Cristalli, 2007) report that this process also happens in Myanmar where the economic embargo imposed by Western countries (with the exception of South Korea) has left regional economic powers without competitors and free to impose their own terms of business.

For instance, the Indonesian Comfeed has its own poultry integrated system already established, and the Thai CP has founded the branch “Myanmar CP Livestock Corporation” by means of foreign direct investment (FDI). Both produce a large amount of poultry products. These types of transnational agro-business companies are export oriented and are supported by government policies. The CP Company, which has a stake in all the SE Asian countries emerging markets, is the world’s biggest feed producer. This behavior mirrors the ambition of Thailand, home of the CP Company, which aspired to become the “kitchen of the world” before being hampered by the H5N1 HPAI epidemic (Delforge, 2004).

In the Northern Shan State of Myanmar FDIs are made by Chinese investors who establish export oriented poultry businesses. The Myanmar Shan State has an intense poultry product exchange with the bordering Chinese Yunnan State. Poultry breeders are imported into Myanmar together with equipment while live poultry and eggs are exported to China (Cristalli, 2007[1]).

3.1.3. Technical support to Myanmar

Paragraph 3.2.2 shows that even with good reasons for controlling and limiting growth of the poultry population, social and economic drivers are making it larger as part of a continuing trend. If these facts are combined with the consideration of the spread of the H5N1 HPAIV

[1] Unpublished consultancy report, see bibliography for reference.

Asian lineage as reported in paragraph 3.1.1, it is arguable that the international community should have an interest in supporting AI control in Myanmar. Yet the country has received little or no international support in comparison to other SE Asian countries. At time of the doctoral research start, this represented the first attempt to help Myanmar in addressing AI and developing control measures despite the FAO policy that was publicly laid down as reported in paragraph 3.1.1.

3.1.4 History of AI outbreaks in Myanmar as of July 2011.

The history of outbreaks is reported in Table 1.

Table 1: History of H5N1 outbreaks in Myanmar (OIE, 2011)

Year	Month	Outbreak description	Epid. Wave
2006	Mar	New cases. 48 countries notify new outbreaks (29 in poultry).	First
2006	Apr	New outbreaks in poultry	First
2007	Feb	New case in poultry	Second
2007	Ma	New consecutive outbreaks in poultry	Second
2007	Apr	New cases in domestic and wild birds	Second
2007	Jun	New H5N1 outbreak in poultry	Second
2007	Jul	New cases	Second
2007	Aug	New H5N1 cases in poultry	Second
2007	Oct	New cases	Second
2007	Oct	Declaration of depletion of all outbreaks	End of epidemic
2007	Nov	New outbreak	Third
2007	Aug	4 New cases in poultry	Third
2008	Apr	Declaration of absence of HPAI	End of epidemic
2010	Feb	Notification of an outbreak in several avian species	Epidemic of 2010
2010	Feb	Cluster of cases in poultry	Epidemic of 2010
2011	Jan	Notification of a HPAI outbreak	Epidemic of 2011
2011	Jan	New cases of HPA	2nd epidemic of 2011
2011	Feb	New case	

3.1.4.1. Description of epidemic waves

Although surrounded by HPAI H5N1 infected countries, Myanmar remained free for two years longer than its neighbors. After the index case (first case), 6 epidemics occurred. Each had a distinct origin, dynamic and follow-up. Epidemics are described below and tabled in Table 2.

The **first epidemic wave** occurred in Central Myanmar. The index case is dated 8 March 2006 and gave rise to a cluster of outbreaks across 13 townships. The epidemic started in the Sagaing Division and spread to the Mandalay Division infecting 545 poultry farms (mainly backyards). Myanmar was declared to be H5N1 free on 4 September 2006.

The **second epidemic wave** occurred in Lower Myanmar between 28 February and 27 March 2007. It generated a cluster of outbreaks which spread across Yangon Division and Bago Division and infected the Mon State. Despite control measures that were implemented, 76 farms were infected.

The **third epidemic wave** occurred at the end of 2007 in the Eastern part of the large Shan State. The virus circulated in backyard flocks. The country was declared to be H5N1 free on 21 April 2008.

Table 2: Synopsis of H5NI HPAI epidemic waves occurred as of July 2011.

Outbreak	**Area**	**Location**	**Date**	**Dead / Killed**	**Infected flocks**
1[a] Wave (2006)	Central Myanmar	13 Townships in Sagaing and Mandalay Division	8 Mar - Apr 2006	650.00	545
2[a] Wave (2007)	Lower Myanmar	5 Townships (Yangon Division)	28 Feb - 27 Mar 2007	65.812	23
Follow up		Insein Township, Yangon	22 May 2007	986	1
Follow up		Bago Township, Bago	2 Jun 2007	960	1
Follow up		Thanbyuzayat township, Mon State	21 Jul 2007	950	2
Follow up		Letpatan Twnship, Bago	28 Jul 2007	5.213	1
Follow up		Thanatpin township, Bago	19 Oct 2007	43.312	48
3[a] Wave (2007)	Oriental Myanmar, Shan State	Kyaing Tong Township	18/11/07	33.586	Backyard
Follow up		Mong Phyat	23 Dec 2007	1.056	Backyard
1[a] Outbreak of 2010	Lower Myanmar	2 townships (Yangon Division)	03 Feb - 16 Mar 2010	2.581	3
2 [a] Outbreak of 2010	Central Myanmar	Monywa, Yinmarbin Townshisp (Sagaing Division)	26/03/10	2.900	2
1[a] Outbreak of 2011	Lower Myanmar	Sittwe (State of Rakhine)	06 Jan 2011	800	

(OIE, 2011; FAO, 2011; Dr. Su Mon, personal comunication, 2010)

Epidemics of 2010. Two unrelated clusters of outbreaks occurred in 2010. The **first** involved 3 farms in the Townships of Mayangone (9 February 2011) and Mingalardon (19 February 2011), to the West and North of the capital district respectively. Stamping out measures were implemented. The **second** occurred in the Thayetkan Village of Yinmabin Township in the District of Monywa of Sagaing Division on 1st March (Central Myanmar). In 2012 a total of 5.491 heads of poultry were officially reported to be dead from H5N1 HPAI or killed as a part of stamping out (FAO, 2010).

Epidemics of 2011 (until end of July). One outbreak occurred in a poultry farm in the Rakhine State as of the beginning of January. Between 6 and 26 January, 6 outbreaks also occurred in the Sagaing Division.

At present, 3 epidemic waves occurred between 2006 and 2007, no epidemics were reported in 2008 – 2009, 2 epidemics occurred in 2010, and 2 epidemics occurred in January 2011. The overall accounting of dead / killed poultry amounts to about 935.000 heads distributed in and around 650 premises (including backyards and poultry farms). The chicken breeds involved were both indigenous and commercial hybrids.

3.1.5. Control Measures adopted in Myanmar.

After the occurrence of the first three epidemics in 2006 – 2007, post outbreak surveillance measures were put in place by the Myanmar

Veterinary Service in the areas deemed more at risk (risk based surveillance). Areas were prioritized based on:

- Poultry density
- Diffusion of traditional duck farming
- Presence of live bird markets (LBMs)
- Presence of wetlands and swampy areas
- Previous history of AI
- Areas bordering with areas of other countries in critical epidemiological situations
- Areas with high production and exportation of live poultry and poultry products.

H5N1 HPAI surveillance has been carried out with various methods. From 2006 to the present the following techniques have been used alone or mixed:

- <u>Farmers' reporting</u> or passive surveillance
- <u>Syndromic surveillance</u> (based on clinical symptoms),
- <u>Participatory surveillance</u> (active surveillance based on interviews with key informants).

- Information was also sought and generated through sampling and collection of specimens and in particular by means of serological surveillance for the H5 subtype. Samplings were carried out which generated fragmented data and uncompleted pieces of information, especially about the informal non intensive sector. As of July 2012 a centralized effective system for archiving, storing and analyzing sampling data with a consistent approach was not in place.

3.1.6. Objective of the doctoral research

The general objective of this doctoral research is to test if a surveillance scheme can generate information and knowledge on the dynamic of the H5 AIV subtype in Myanmar. The surveillance scheme taken into consideration and tested was specifically tailored to the Moeyingyi wetland. This area is a very complicated case and is considered one of the more challenging epidemiological situations for a test.

The surveillance scheme trialed corresponds to the protocol of the field epidemiology study implemented.

The present research pursues the following specific objectives:

(1) to trial a surveillance scheme which can produce a reliable and valid picture of AI infection in the domestic population of the Moeyingyi wetland,

(2) to increase the knowledge on the epidemiology of AIV circulating in Myanmar with particular focus on the Moeyingyi wetland which, being considered an epidemiological hot spot and an AIV reservoir, is believed to offer an interesting model,

(3) to make the information generated by the study available to the local veterinary services in order to stimulate the development of tailored and sustainable control measures.

3.2. Problems and delays

The doctoral research had a delayed start in comparison to the envisaged date (2008) due to the unfortunate occurrence of the Cyclone Nargis which hit Lower Myanmar in 2008. Severe damage to the country's infrastructures (including the veterinary services) hampered any activity not directly concerned with humanitarian aid. Once veterinary services were restored to basic operative capacity, they were fully committed to provide assistance with restocking. A one year delay on the completion of the doctoral research was then requested and agreed to.

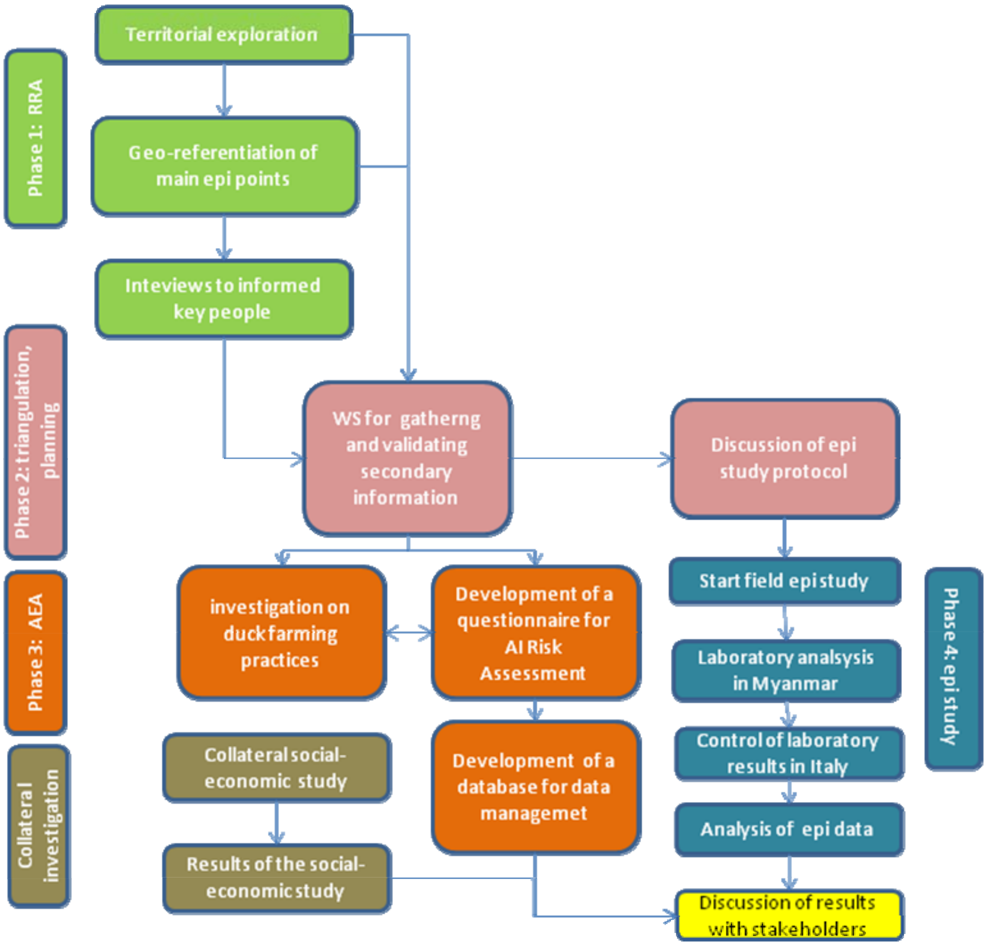
In its final stage the research had to deal with the inextricable bureaucratic perversity of the shipping authority of Myanmar which oversees and releases permissions for the shipment of goods out of the country. It is necessary to mention that countries have a shipping authority which deals with shipping companies and couriers. The only interlocutors accepted are representatives of these companies. For reasons not entirely understood, the shipping authority of Myanmar interpreted the IATA (International Air Transportation Association) guidelines classifying the goods to ship as viruses instead of diagnostic sera. This interpretation imposed reorganization of the shipment as if the 1000 serum samples were 1000 dangerous virus cultures. This interpretation dramatically increased the price of shipment and delayed it. The shipment of samples was necessary to verify the concordance of the locally performed analysis with those of the Italian OIE reference laboratory for AI. The delay accounted to 6 months and posed a serious problem for the proper refrigerated conservation of the samples in Myanmar.

4. MATERIALS AND METHODS.

The present chapter describes the method of all the four phases outlined in Diagram 1. Distinction is made between phases 1, 2, and 3, and phase 4. The first three phases are considered preparatory and describe the method followed to enable the execution of the epidemiological study, which is phase 4. Preparatory phases hinge upon development and management disciplines classifiable as “soft” or “medium hard science” while the epidemiological study is based on “hard science” as originally defined by Norman (1967) and is thus more relevant to and consistent with the expected doctoral research results.

The implementation of a scientific protocol in a difficult environment and context is challenging because (i) an incomplete knowledge of the reality may result in the risk of implementing protocols with internal consistency but irrelevant to the context, (ii) managerial capacities are required for forecasting difficulties, planning, staffing and motivating others in order to assure the implementation of the study. These difficulties may be reduced if the research is implemented following sound steps through preparatory phases. These phases rely on tools used by the development disciplines.

Diagram 1: Phases of the research



4.1. Way of Working (WoW)

The research has been enabled by the endorsement of the Italian Ministry of Health to an Initiative of the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe). It was developed between IZSVe and the managing structure of the veterinary services of Myanmar: the Livestock Breeding and Veterinary Department (LBVD). The research has been managed jointly. The writer was the technical responsible for research and acted as project manager. The counterpart representing Myanmar was the head of the department of animal health of LBVD. A team of LBVD veterinarians was created to support the field activities. Several of external collaborations and strategic relationships with local authorities (internal visa and approved agenda of movements) were necessary. Some project activities as the drafting of the epidemiological protocol and double laboratory check on a sample of specimens were carried out in Italy while the remaining activities were fully implemented in Myanmar. The area of investigation was identified by LBVD (Phase 1). The study protocol was developed and fine tuned and approved subsequently (Phase 2). Stakeholders were involved with a participatory approach and adopting a flat management system (Stockbridge, 2008) which included others in the decision making processes. For instance, the FAO started operating on HPAI control in Myanmar in 2008. The office was managed by the FAO Chief Technical Advisor (CTA) for HPAI, whose collaboration was sought. The present research has pursued complementarities with FAO activities and, in practice, one of the sampling rounds of the epidemiological study (phase 4) was funded by FAO and was implemented jointly with FAO staff. The collateral socio-economic study (see Diagram 1) was implemented by the International Non Governmental Organization (INGO) Winrock International and funded by the FAO.

The writer was always present during field activities in the agreed position of main researcher and manager of the wider project.

4.2 Methods of the preparatory phases

4.2.1. Method of phase 1: Rapid Rural Appraisal (RRA)

The first survey of the proposed location corresponded to the territorial exploratory phase. The reference method used is the RRA (Chambers, 1991). Although it has been labeled with negative connotations it is still considered a necessary step for projects located in complex and not sufficiently known areas. It was based on observations done during transect journeys across the territory, use of experts' insights and interviews to key informants. Interviews were done with relevant farmers, local authorities, and local veterinary authorities. The selection of interviewee was driven by the opportunity of finding duck farmers along the route of the transect investigated. In order to allow flexibility and to avoid any bias of imposed agendas interviewees were not informed before. The visit to one hatchery was also a last minute decision. The main epidemiological points were geo-tagged using a Garmin ® receiver (Garmin LTD, Bangkok, Thailand) and its bundled software. The level of details of the Map was very low. No better alternatives are available but the web based Google Earth **(**© 2012 Google) freeware. Maps were finally sketched and the whole research area was subdivided into hypothesized smaller agro-ecosystems with common features. The implemented RRA was characterized by a high cost effectiveness ratio and was carried out in two days. This phase was implemented by the writer who was supported by LBVD as translators and facilitators.

Philosophy of the method: survey tool of development sciences. Highly cost effective method based on experienced consultants' insights (Chambers, 1991).

Timeframe: February 2009

4.2.2. Method of phase 2: triangulation of information, motivation of stakeholders.

The information collected during Phase 1 was consolidated and compared with other secondary information obtained from informed people (see list below) and authorities. A workshop was organized in Yangon with the purpose of introducing the research to all the interested parties, to refine the analysis of stakeholders, to motivate participants, and to share the draft of the epidemiological study. Participants were requested to present some relevant information according to their background and current position. Information was triangulated and discussed during plenary sessions.

The workshop was attended by:

- One LBVD PhD student
- The veterinary authorities of the four townships of the study area
- The veterinary authority of the Bago East Division
- The owner of the biggest duck hatchery of the study area
- One representative of duck farmers
- The FAO CTA for HPAI and other FAO vet officers
- The CVO of the Myanmar Veterinary Services
- The staff of the LBVD
- The Central Veterinary Laboratory (CVL) staff

Philosophy of the approach: participatory tool of development approaches (Chambers, 1994)

Timeline: April 2009

4.2.3. Method of phase 3: Agro-ecosystem Analysis (AEA).

The territory was then investigated in depth using the approach of the agro-ecosystem analysis (Comway, 1985). The purpose was to increase the knowledge on the setting and at the same time provide the information necessary to plan sustainable disease control measures.

The concept agro-ecosystem entails that an ecosystem governed by the law of nature is put under the human control for production of food and / or non food items. The ecosystem thus becomes a cybernetic system with a scope. The elements which constitute the agro-ecosystem are both natural and human (Pretty, 1995). Given the close interdependence of humans with the environment and given the many interactions between humans and the environment, participatory methods are often used during the AEA, which is then defined as "participatory" AEA. Tools like focus group discussions (FGD), seasonal calendars, and Ranking and Matrix Scoring (Laws, 2003) have been used for the present AEA. The scope was to obtain a "good enough picture" of the reality investigated and to get detailed information about the agriculture, the duck sector, and the duck value chain.

Different aspects were targeted. An AEA was pursued to confirm that the wetland area was comprised of four smaller areas with similar environmental characteristic. These areas were used to subdivide the studied area into smaller fractions which became the strata of the sampling plan of the epidemiological study. These areas were mapped out and land demarcated based on territorial natural features or manmade infrastructures.

The knowledge the local agro-ecosystem was necessary to identify the possible risk factor for the transmission or perpetuation of the H5N1 AIV in the area.

Philosophy of the approach: Development tools largely based on indirect observation and interpretation of local people (Narayanasamy, 2009).

Timeline: April 2009

4.3. Method of phase 4: epidemiological study

The epidemiological study is the evaluation of the innovative surveillance scheme proposed that is trialled in the field. In essence, the epidemiological study is based on sampling activities, laboratory analysis, and basic descriptive epidemiology of laboratory results. Some aspects of data analysis such as the survival analysis and the specific analysis are additions to the proposed surveillance scheme and are executed for evaluating it. It is not expected that the same analysis is carried out when the surveillance strategy is replicated by LBVD in other places.

4.3.1. Rationale behind the type of the epidemiological study.

A previously implemented qualitative risk assessments executed by the veterinary services warned about the existence of an AI persistent risk prone area corresponding with the Moeyingyi wetland. The identified area was even hypothesized to be the main AIV reservoir of the country and also a reactor-cradle for new subtypes (Sunn, 2009)

It was decided to perform an epidemiological investigation with a perspective design.

This choice was driven by:

1. epidemiological reasons linked to the need to effectively investigate the Moeyingyi case,
2. the low performance of the commonly implemented large scale routine surveillance in domestic duck populations.

4.3.1.1. Epidemiological reasons

The lack of pre-existing epidemiological data led to choosing a type of investigation based on newly generated data and information rather than relying on secondary ones. The urgency to provide answers to the LBVD provided the motivation to plan a sound protocol which could produce the necessary information for planning control and eradiation measures. The other option of epidemiological study type was the case-control model which was ruled out due to the lack of preliminary sound information about disease / infection occurrence. It was actually impossible to identify either cases or controls.

4.3.1.2. Reasons linked to low performances of duck surveillance schemes

In practice it was necessary to demonstrate that the model tested was able to use financial resources in a cost-effective way and that it was replicable and scalable to other geographic areas. It had therefore to be cheaper, more effective and more easily executable in comparison to previously implemented schemes. It is acknowledged that routine surveillance in domestic ducks has a low sensitivity (Barber *et al.*, 2011) and that, based on other investigation concurrently carried out in Myanmar, virological surveillance has a high financial cost, and requires relevant human resources but gives unsatisfactory results. On the contrary serological surveillance showed many H5 positive results which didn't match with virological finding. To solve this problem, the longitudinal surveillance proposes an approach based on the following points:

- identification of a cohort of negative farms,
- follow up of these farms over time, reducing the number of investigated cases in order to reduce costs,
- focus on serological surveillance,
- promptly identify the serum - converted cases
- critical interpretation of the number of positive samples and of the Ab titres in order to estimate the time of infection and the possibility of virus shedding (described in paragraph 4.3.2.1)
- concentrate the effort of virus isolation only on those farms which have a high probably of being in the circumstance of viral shedding (described in paragraph 4.3.2.1).

This approach would reduce costs for executing “blanket” virological surveillance with low sensitivity and would concentrate laboratory analysis on specimens likely to be infected.

4.3.1.3. Type of epidemiological study adopted

The epidemiological investigation scheme adopted is the **prospective study** where the **sampling unit** is defined as the small scale commercial oriented duck farm and the **positive case** is defined as the sampling unit which has at least one positive duck. The tests used are

the Haemoaglutination Inibition (HI) test and the virus isolation test (VI). Laboratory methods are described in paragraph 3.4.4.

The longitudinal perspective allows for monitoring the selected units along the time in subsequent surveys. Double checks and recording of repeated observations are also necessary to increase the validity of data. Longitudinal surveys allow investigators to record and monitor risk factors and environmental changes and to evaluate their possible role as determinants of the positive cases. Weather conditions and climate characteristics and environmental changes across the different seasons are among the most relevant risk factors for infections. This evaluation is made possible only by longitudinal designs with repeated surveys. The longitudinal cohort study model was adopted. The negative cohort of sampled units was identified by means of a cross sectional study. This model allowed for:

(i) Assessment of the incidence of infection in negative farms during the different phases of the production cycle and across different seasons (these environmental factors are automatically considered in the study) ;

(ii) Registering and measuring of risk factors identified during AEA (as described in paragraph 4.2.3) and their variations.

The **time interval** between surveys was variable. Intervals were planned to fit the availability of farmers who are often busy in other agriculture activities. They were also planned in a way to follow in time some agriculture phases. For instance, some important moments of the duck production cycle (housing, sale, moulting) and some major climatic events (start of rainy season, dry season) or agricultural phases (harvest, sowing, rice seedlings' transplantation, etc) could represent a risk factor. Following these principles, intervals among samplings ranged between 30-60 days.

4.3.2. Epidemiological study protocol

A negative cohort of duck farms was identified by means of a **cross-sectional investigation** of **40 duck farms** which were randomly selected from a sampling frame estimated at **1769 duck farms**. The number of farms sampled responds to principles of opportunity rather than to an accurate sizing. Limiting factors were the accuracy of the sampling frame and the scarcity of human and financial resources.

Farms were distributed in 4 strata and their number per stratum weighted based on the number of total estimated farms active in each stratum. Duck farms varied in size, housing flocks ranging from 300 to 3000 heads. They all were market oriented. Domestic duck population at the beginning of the study amounted to approximately **3.823.344 ducks.** This precise value is the sum of all the township poultry censuses which were asked to township veterinarians and consolidated during the participatory workshop carried out in Phase 2.

In case the selected farm was not found or reachable, the replacement was done by selecting a farms with similar characteristics in the same village.

The negative cohort was identified based on laboratory testing (described in paragraph 4.3.4.). The negative cohort had to be followed for a 7 month time (September 2009 – March 2010) which corresponds to the seasonal egg production period for ducks. Sampling surveys were scheduled in September, November, and December 2009 and in January and March 2010.

Once a farm becomes positive it exits the negative cohort and leaves the study. Only negative farms are continued to be sampled. In case the cohort becomes too small for enabling the survival analysis (described in paragraph 4.4.1), new negative farms can be introduced into the studied group. If necessary, new negative farms can be identified by means of an additional cross-sectional study.

The number of sampled birds within each flock (see paragraph 4.3.3) was calculated to enable the detection of a positive bird with a confidence of 95% assuming a within-flock prevalence of 9% and flock sizes ranging from 100 to 3000 birds. In each farm sera plus tracheal or cloacal swabs were collected from 30 randomly selected birds.

4.3.2.1 Additional attempts to identify AIV based on critical serological investigation

The interpretation of serological results was used to identify some farm infected recently and thus more likely have ducks which shed virus. This method was implemented as described in paragraph 5.6. The implementation of the present activity was done to fill the gap between serological positive results and virological negative ones. Three farms were selected based on the following principles:

- Negative at previous sampling,
- Positive Ab titres against the H5 subtype but at low log,
- Low Percentage of positive sera on the total collected and tested

4.3.3. Sampling operations and samples' management

Sampling operations were carried out by a team comprised of veterinarians of the epidemiology department of LBVD and the township veterinarian supported by some *extension workers*. Sampling activities were always overseen by myself.

In each farm, the sampling operation protocol envisaged:

- Recording the GPS coordinates of the farm house,

- Random collection of 30 specimens for serological and virological testing. Blood was collected for serological testing, cloacal and tracheal samples for virological testing. In case tracheal samples could not be collected then oral-pharyngeal samples were their replacement. Samples for virological testing were stored in Falcon tubes. Every five samples were pooled and antibiotated virus transport medium was added. The transport medium was composed of Phosphate Buffered Solution (PBS) containing penicillin (10.000 IU/ml), nystatin (5.000 UI/ml) and gentamycine sulphate (250 μg/ml).

- Filling of questionnaire by means of asking questions and using direct observation on farming practices and AI risks. The questionnaire used required several fine tunings and was finalized only after a cycle of testing and reassessment. After this process it was then tailored to the responding capacity of local people and to the time available. The English draft of the questionnaire used is reported in Box 1 .

Box 1: Questionnaire for risk factor measurement

Interviewer………………………

Date..……/………/………

Questionnaire for Duck Farmer

Basic information

Farm name..owner name…………………………………......

Farm address………………………………………….……...Tel………

Owner address………………………………………………..Tel………………

Experience.......................... year/ month.

Total population …………

Meat type ☐

Layer type ☐

Farm management

1. Household characteristic

Back yard ☐ intensive ☐ semi-intensive ☐

2. Source of flock:

Address or Location……………………………………………

3. Which ages of ducks you start to farm?

DOD ☐ one moult ☐ two moult ☐ three moult ☐

4. Buying price of duck:

…………………………………………………………………

5. Duration of flock keeping:

Age: 6-12 months ☐ 1- 2 year ☐ 2-3 year ☐ over 3 year ☐

6. Age of laying start:

Age 4 months ☐ 6 months ☐ more than 6 months ☐

7. Where are you setting your ducks? How far?.......................

canal ☐ pond ☐ lake ☐

8. Any health problem in the flock?

…………………………………………………………..

9. How do you sell your eggs:

Self ☐ Via Dealer ☐

10. Selling price duck egg:

Average…………kyats

11. How do you bring your eggs?

basket ☐ egg tray ☐ wooden box ☐

12. What do you use as duck bedding?
rice straw ☐ saw dust ☐ rice husk ☐

13. Do you reuse egg container and bedding? Yes ☐ No ☐

14. Supplementation of feed:
Yes ☐ No ☐

15. Kind of feed:
…………………………………………………………………

16. Source of feed:
…………………………………………………………………..

17. Is there poultry farm within 1km radius from this farm?
Yes ☐ No ☐

18. N° of farms ☐

19. Type of poultry
………………………………………………………………………………..

20. Do you know HPAI? Yes ☐ No ☐

If ‘**yes**’, please write your opinion
……………………………………………………………………..

Signature of interviewer
………………………..

4.3.4. Laboratory analysis

Laboratory analyses were carried out at the Central Veterinary Laboratory (CVL) also mentioned as Yangon Veterinary Diagnostic Laboratory of Myanmar located in Insein, Yangon. CVL received training from the FAO and JICA (Japanese International Cooperation

Agency) on OIE standard laboratory techniques. The increased capacity and experience of CVL was positively evaluated on the base of the outcome of the laboratory proficiency test carried out on the AI routine diagnosis. This test, regularly carried out by the OIE/FAO regional reference laboratory, allowed for reliance on the CVL for HI against the H5 subtype antibodies (Ab) and for virus isolation in embryonated eggs.

4.3.4.1 Serological test

Serum samples were tested using the haemagglutination inhibition (HI) test to detect Ab against circulating H5 virus. Tests were performed at the national Veterinary Laboratory. The antigen used in the HI test was prepared from a virus isolated from a chicken that died from HPAI H5N1 during the second HPAI epidemic, which started in February 2007 in Lower Myanmar. HI titres were considered positive if there was inhibition at a serum dilution of 1/16 (2^4) or more against 4 haemoagglutinating units of antigen. The procedure of the HI test followed the OIE standards even if the H5 inactivated virus did not originate from an OIE standard laboratory and was not yet formally classified at time of testing. No preliminary serological testing for searching Ab against Type A AIV were performed in order to save money and in agreement with the local laboratory practices. Serological surveillance carried out only by performing HI against the

H5 subtype is considered an acceptable practice in SE Asian AI H5 subtype endemic or at risk countries (Desvaux *et al.*, 2012).

4.3.4.2 Virological test

Virus isolation was carried out by inoculating pools of 5 swabs in embryonated specific antibody-negative (SAN) chicken eggs at the 9^{th}-11^{th} day of embryonic development. Eggs were incubated at 37°C for 4-7 days. The test was carried out as prescribed by the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2009). Pools were created after their collection in the field and contained in vials with a transport medium with added antibiotics. SAN embryonated eggs were provided by the Comfeed breeding farm which did not vaccinate hens against any AIV and carried out routine test for AIV. The use of SAN embryonated eggs is accepted by the OIE as an alternative to Specific Pathogen Free (SPF) commercial eggs. Standard OIE reagents were made available to CVL by FAO and/or OIE regional reference laboratories such as AAHL (Australia) and the national Tai reference laboratory.

The molecular diagnosis performed locally was a practice not yet considered sound and anyway more expensive than egg inoculation.

4.3.4.3 Concordance of locally performed tests with OIE standards

A sample of selected sera with unusually high Ab titres against the AIV H5 subtype and some specimens for virological investigation highly suspected to be H5N1 HPAI positive were also tested at the OIE/FAO reference laboratory for Avian Influenza and Newcastle Disease hosted at IZSVe. Sera were tested for anti Type A Ab and for H5 Ab. When results were compared with the CVL output any statistical test for concordance appeared redundant due to the complete overlapping of results. The Ab titres which were apparently very high at the CVL testing were also tested at IZSVe to clarify the issue and finally resulted even higher. The sera shipped to IZSVe were collected from the same flock of the virological samples and in particular from farms coded DTC0001, DTC0002 and DTC0003 which are described in paragraph 5.6.

Virological tests were carried out by means of RT-PCR. All the samples were screened by Real Time RT-PCR (rtTT-PCR) targeting the Influenza A specific M gene (Spackman *et al,* 2002). Positive samples were typed by rtRT-PCR protocols specific for H5, H7 and H9 subtypes (Monne *et al,* 2008). Molecular diagnosis was followed by virus isolation in embryonated SPF eggs (OIE, 2009). While the CVL in-egg testing always gave negative results, some samples showed RT-PCR results positive for an unidentified H subtype of Type A Influenza virus. Even organs corresponding to HI highly positive sera against H5 Ab gave negative results from the H5 gene RT-PCR and were negative at the embryonated eggs inoculation.

4.4. Epidemiological analysis

Descriptive epidemiological parameters were updated in real time during the implementation of the epidemiological study in order to control the study and allow for timely decisions and corrective actions. Descriptive analysis is done by accounting for prevalence and incidence data. These parameters were broken down per strata. Descriptive analysis was also performed on the number of positive samples per farm. Survival analysis was performed at the end of the field activities.

4.4.1. Survival Analysis

The objective of the analysis is to evaluate the time necessary for seroconversion of the group of farms investigated.

The parameters used are the "*follow-up*" period, the "*population at risk*" at the beginning of the observation period and the "*event of interest*" which is the seroconversion. In most of the cases the "*non event*" is the exit of the observed farm due to the sale of the flock or the migration to other locations.

The follow up period is defined by a date of start and a date of end of observation. The date of start is the date of first sampling. The date of end of observation is that of serum-conversion or the date of exit from the study. The population at risk is composed of all the farms investigated and thus encompasses farms entering the study with both the first and the second cross sectional survey. The event of interest is serum-conversion. The non event corresponds, in most of the cases with the exit from the study due to the stop of business (censored); only one of the farms enrolled in the study at the first cross sectional study arrived end of the study uninfected. The representation of the time of seroconversion is represented using the Kaplan Mayer curves (Klein *et al*, 1992). Curves were comparing using the Wilcoxon test.

5. EXECUTION OF THE EPIDEMIOLOGICAL STUDY

This chapter describes the field activities implemented, reported by survey round. The date of start is September 2009. Field operations were completed in April 2010. In total, 80 different farms were involved. 40 entered the study at its start and another 40 were added in November 2009.

The sampling of duck farms is outlined in Figure 1 and described in the following paragraphs.

As described in paragraph 5.3 the introduction of new negative farms to restore a minimum number of duck farms to the cohort was necessary for attempting a statistical representativeness of the studied area. Five sampling rounds were implemented overall. Farms introduced in November were sampled a maximum of three times. 16 farms left the study, but none of them due to causes connected with health, thus not for AI causes. The farms which left the study represent the "non event" or the "censored" cases of the epidemiological survival analysis as described in paragraph 4.4.1. Most of the farms which left the study (15 out of the 16) stepped out in the period between January and March.

5.1 First sampling (September 2009)

The study started with a cross sectional survey on 40 farms. Field activities were implemented between 9 and 12 September 2009. Blood samples for serological testing and cloacal swabs for virological testing were collected. Cloacal specimens were collected by means of cotton swabs meant for human use because the proper material was not available. Pools composed of 5 swabs were prepared as described in paragraph 4.3.3. Together with the biological specimens, information about the farm and about the H5N1 HPAI infection risk factors was collected. In particular, the husbandry and management practices were investigated. Each duck farm was geo referenced. Despite the envisaged protocol that also included the collection of samples from the upper respiratory ways (tracheal or pharyngeal), the unfortunate lack of the proper material hampered their execution.

5.2 Second sampling (October 2009)

The 18 farms which yielded negative results at the first sampling / testing were sampled again between 11 and 16 October 2009. During this survey virological sampling was prevented by a lack of swabs.

5.3 Third sampling (November 2009)

Between October and November one duck farmer stopped his business and left the study. Sampling was carried out between 17 and 21 November 2009. Due to the reduction of the number of farms actively investigated (still negative) a second cross sectional study was done and 40 new randomly selected farms were sampled. 18 out of the 40 newly investigated farms yielded negative results and entered the cohort of negative farms. From this moment the number of negative farms included both the newly introduced negative farms as well as those remained negative out of the original cohort, constituting the a group 29 farms.

The second cross sectional investigation was made in collaboration with the FAO, which supported the sampling with some staff recruited for other newly designed AI sampling activities interestingly called “National Duck H5N1 Cross-sectional Study," extending the surveillance scheme that was trialed to other locations in the country.

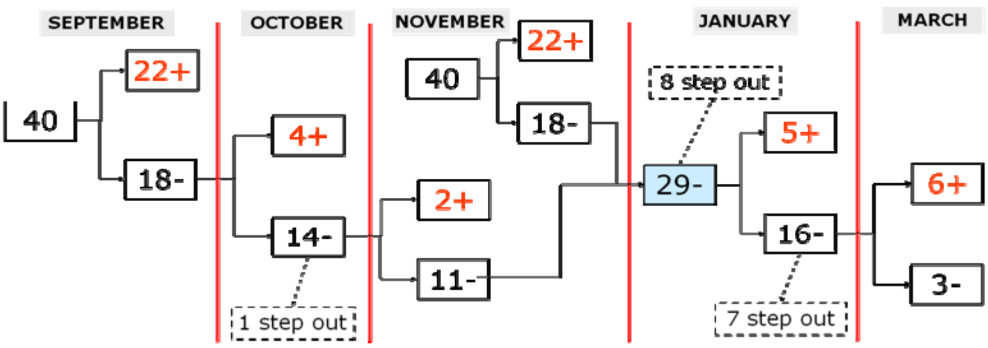
5.4 Fourth Sampling (January 2010)

The remaining 29 negative farms were visited in January 2010, but 8 farms had stepped out their business and sold their flocks so only 21 farms were addressed.

5.5 Fifth Sampling (March 2010)

7 out of the 16 previously negative farms had stopped their business. All farms in the “lake” stratum became infected.

Figure 1: Scheme of sampling following the longitudinal design.



5.6 Targeted sampling (additional attempt to virus isolation)

Three duck farms located in the canal stratum were addressed by the intensive sampling strategy for increasing the probability of isolating the H5 AIV subtype. The farms identified were:

1. Farm DTC003:
    1. Ducks housed: 600 ducks for 9 months and 160 ducks for 4 months.
    2. Ducks sampled: 460 / 600 and 160 / 160 (tracheal and cloacal samples)
2. Farm DTC002:
    1. Ducks housed: 300
    2. Ducks Sampled: 286 / 300 (tracheal and cloacal samples)
3. Farm DTC0001:
    1. Ducks housed: 500
    2. Ducks sampled: 100 / 500 (tracheal and cloacal samples)

6. FINDINGS OF PREPARATORY PHASES: THE CONTEXT

The project phases described in paragraph 4.2 produced information about the environment. This information was necessary for (1) enabling the implementation of the epidemiological study and (2) informing the territorial AI control strategy.

In particular, this information was used to frame the epidemiological study in strata. The present chapter analyses the studied area first by issue (environment, wildlife, poultry sector etc) in paragraph 6.1 (and sub-paragraph) and then by homogeneous areas called agro-ecosystems or strata in paragraph 6.2 and its sub-paragraphs.

6.1 Geographical information of the studied area

The area studied corresponds geographically to the Moeyingyi area and its swampy surroundings. This is called generically the Moeyingyi Wetland area. It is located in the Bago East District and is around 100 km to the northeast of the capital. The wetland falls into four townships. The Moeyingyi Lake is in the middle and has a minimum surface area of 16 hectares. The lake was acknowledged officially as a Ramsar protected area in 2005 (Ramsar, 2005) and is currently the only one recognized in the country. It is home to 125 wild resident and migratory bird species. With 3000 laying duck farms housing around 2-4 million head (Aung Khin, 2009), the area immediately surrounding the lake is the largest duck production and reproduction area of Myanmar. Indeed, 60% of the country's duck population is hatched in the Moeyingyi area, is raised traditionally, and is distributed all across the country. Ducklings, eggs, embryonated food eggs, spent layers, and drakes are produced from the Moeyingyi area. The area to the south of the lake is crossed by the manmade Bago-Sittaung canal, which flows across the Waw and Thanatpin townships. The canal is a fluvial infrastructure built by the English colonists with the double purpose of channeling groundwater and enabling transportation of goods. It is around 60 km long and connects the Sittaung and Bago rivers.

The area is characterized by secondary and tertiary canals which feed the Bago-Sittaung canal. The area surrounding these canals is classified as rice cultivated land whose flooding is governed through gates which release the water outflow from the lake through the artificial earth bund into a network of irrigation canals which deliver water to the fields. The land between canals is cultivated with rice and is periodically flooded during stages of the production cycle.

6.1.1. Domestic duck population

As mentioned before, the population of domestic ducks was estimated with the best possible accuracy, but it ranges widely between 2.000.000 to 4.000.000 subjects due to seasonal variations, market trends, and epidemics. Individual flock size ranges from 100 – 4000 ducks. A rapid assessment showed that flock size is linked to location and thus to the type of management. Differences in size depend on factors such as (i) permanent residency inside the Moeyingyi sanctuary, (ii) settlement in the interior of the earth bund (bank) that borders the south of the lake, (iii) location along the Bago-Sittaung canal or (iv) in areas whose water level varies widely between the dry and wet season.

Ducks are confined during night-time and are conducted to water bodies in day-time. The grazing diet is supplemented with shrimps, broken rice and shells. Reproduction is centralised in 12 traditional hatcheries connected with breeding farms which establish temporary contracts with hatcheries. The local rearing practice requires low external input but also has low biosecurity and is sometimes characterized by promiscuity with other species (swine, chickens, Muscovy ducks, cattle, buffaloes, goats, geese) which, when present, are represented in single or very low numbers.

6.1.1.1 Domestic duck breed

The body is small in size, which can be attributed to the focus on laying. The wide morphological diversity observed within a single flock mirrors a wide genetic constellation which contributes to the potential capacity of the population to respond to the many different external challenges of unfavorable environmental conditions. Personal accounts are inconsistent, and report such stories as commercial cross-breeding with Caki Campbel strains, introduction of Peking ducks from China, and the introduction of Roeun ducks. The personal observation of many tufted looking ducks and of body types very close to Indian Runners supports the hypothesis of genetic diversity.

Figure 2: Morphology of a local laying duck type

Figure 3: Morphological variability within a flock

6.1.1.2 Farming structures

Farms each have similar layouts and duck houses are characterized by having a raised floor at varying heights. The height depends on the water level reached by flood waters.

The farms feature pillar houses built of wood and bamboo. The interiors are divided into two zones. The larger zone houses the duck flock, while the other houses the farmer’s family. The zones are barely separated by a fence (Figure 4).

Figure 4: Bamboo shelter used for rearing mid size flocks. It comprises an area for the farmer family (left) and the flock (right).

The roof is made of a bamboo frame supporting patches of palm leaves and rice straw imported from the Mon State (Figure 5). It requires yearly maintenance / rebuilding. This operation is carried out between April and May, before the start of the monsoon season. The quality of the roof is locally considered an indicator of welfare: the poorer use only rice straw while the richest use palm leaves. Bamboo is considered affordable by all, as it comes from nearby hilly locations. The practical and ingenious engineering of the houses combined with building simplicity and the lightness of bamboo is functional to disassembling and rebuilding, allowing for easy relocations. These houses are weak in appearance but are well suited to frequent movement. Relocating is more frequent within areas whose water level / water availability is more variable. Some areas are left until the flood waters recede while life goes on in other places. The availability of water, rice cultivation and duck farming are linked: it is not hyperbolic to assert that rice cultivation could not be carried out without duck farming.

Figure 5: Roof made of palm leaves on a bamboo frame.

6.1.2. Integration of rice cultivation and duck farming

The only staple starchy food produced, which constitutes the basis of the local diet, is rice. Rice is cultivated with low /null external inputs (chemical fertilizers and pesticides). Rice varieties are mostly local and originate from locally produced seed. Broadcast sowing is practiced in some places while in others seedlings are grown in nurseries and then transplanted. Nurseries are small patches of land where hand sowing is done, and has several advantages (mostly from using less land). Tractors are totally absent but replaced by draft power from buffaloes and oxen (Figure 6).

Figure 6: Plowing by means of animal draft power

Duck flocks are conducted to pasture on harvested rice fields, a reciprocal practice between duck farmers and land owners that benefits both parties. Ducks can feed on mollusks, crustaceans, and insects, and pick up the leftover grains. The local duck breed needs just some commercial feed to supplement to this diet. At the same time ducks shed their droppings evenly and increase soil fertility, while at the same time weeding the field. This integration supports farmers' livelihoods. Ducks are usually conducted to rice fields two times a day: in the morning, between 7.30 and 11.00 (never after 10.30 in the hot season), and in the afternoon between 15.00 and 18.00. The flock grazes for around two hours each time and is kept in the bamboo shelter for the remaining time. The flock is always watched while it is at pasture. Different family members may take charge of the flock. Ducks are conducted in and out of the duck house and to the rice field by means of visual and voice signals. It is interesting to report that farmers use the imprinting of ducklings on colored clothes which are then tied on the top of bamboo poles and used as flags to lead the flock to its destination.

6.1.3 Duck rearing and egg production

The production cycle usually starts when 3-9 day-old ducklings (DOD) are purchased and housed in the duck shelter. Sometimes farmers buy a flock at the start of laying, or even adult birds from other farmers who have stopped their business.

Ducks are reared for table egg production and at the end of the production cycle are finished and sold for their meat. The meat of spent ducks is not highly desired in the market because other meaty breeds are more popular and preferred. "Moeyingyi ducks," as described in paragraph 6.1.1.1, are small and light (1.5-2 Kg live weight.).

In 2009 duck eggs were being sold at an average market price of 85 Kyat (€ 0.06) each. Price fluctuations are reported as one of the main obstacles to duck farmers who complain about the impossibility of affording feed supplements, particularly shrimp meal.

6.1. Description of the strata adopted for sampling

The identified strata have peculiar agro-ecological characteristics as described below. They are and visualized in Figure 1. **Errore. L'origine riferimento non è stata trovata.**

6.2.1 Stratum 1: West Side of the lake, called "roadside"

It lies in the area which extends to the east and west of the road which goes north from Bago Township to Daik U Township. It is parallel to the western shore of Moeyingyi lake, which is low and whose shape varies according to the rainy season. The seasonal changes in water levels naturally irrigate the area for rice cultivation. Duck farms located in this stratum make west-east movements of varying distances according to the extent of flooding. The road divides the stratum into two different areas which have different regimens of rice cultivation. Between the road and the lake shore only one rice harvest per year is done (two cycles are possible on the portion of land closer to the road). To the west of the road, the paddies are flooded by means of artificial systems, allowing for two harvests per year. The number of rice cycles per year leads to the possibility that duck flocks can access pasture in the rice field twice per year after the harvesting.

6.2.2. Stratum 2: Moeyingyi lake, called "lake"

It is occupied by duck farms housed in pillar houses located in the middle of the lake. The stratum is characterized by the presence of a high number of residential and migratory birds. Ducks do not have access to the coastal rice fields (which are cultivated during the dry season) due to the long distance from the houses to the shore.

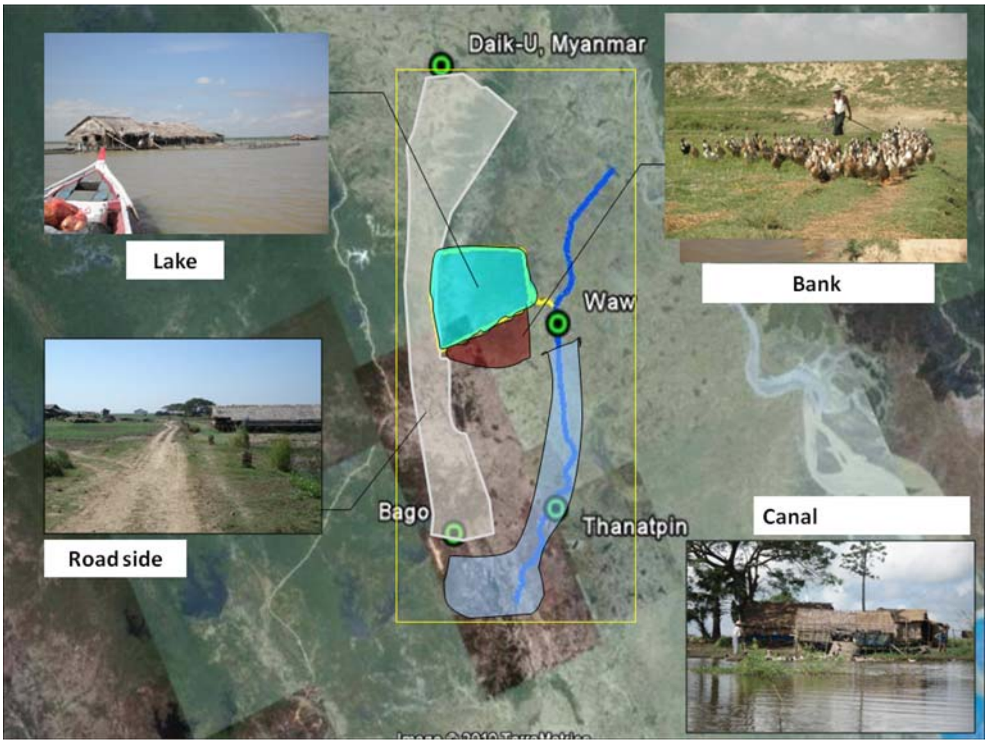
6.2.3 Stratum 3: South shore of the Moeyingyi Lake, called "bank"

The southern shore of the lake is made of an earth bund which prevents the inundation of the land and allows gates to govern water flow into irrigation channels. Farms are located between the earth bund and the rice fields and are residential. Ducks can pasture either in the lake or in paddies according to the time of the rice cultivation cycle.

6.2.4 Stratum 4: Bago-Sittaung canal, called "canal"

It is a densely populated duck farm canal with premises localized on the two banks. Flocks spend a variable amount of time in the canal but are moved to the pastures in the rice fields located in the proximity of the canal. Rice cultivation on the two sides follows a different cycle. Fields located to the north are more easily flooded by the water flowing out from the southern earth bund through the water gates (see stratum “bank”). The water flows from the Moeyingyi Lake southwards. Rice fields located along the northern area of the canal benefit from more water and can produce rice during the summer time which is also called “summer rice”. This cycle requires that sowing is done in mid November and harvest is done in May. After sowing the ducks are banned from pasturing in the fields. Flocks are therefore moved to the rice fields located on the south side of the canal. These fields are devoted to the production of so-called “rainy rice” which is cultivated after the “summer rice”. Sowing is done between May and June and harvest occurs at the beginning of the wintertime during November and December. The two different cultivation cycles rely on two autochthonous varieties which are different for phenology, organoleptic characteristics and morphology. Ducks rotate between the harvested rice field pastures according to their cycles and then move continuously from side to side along the canal.

Figure 7: Different strata of the Moeyingyi Wetland



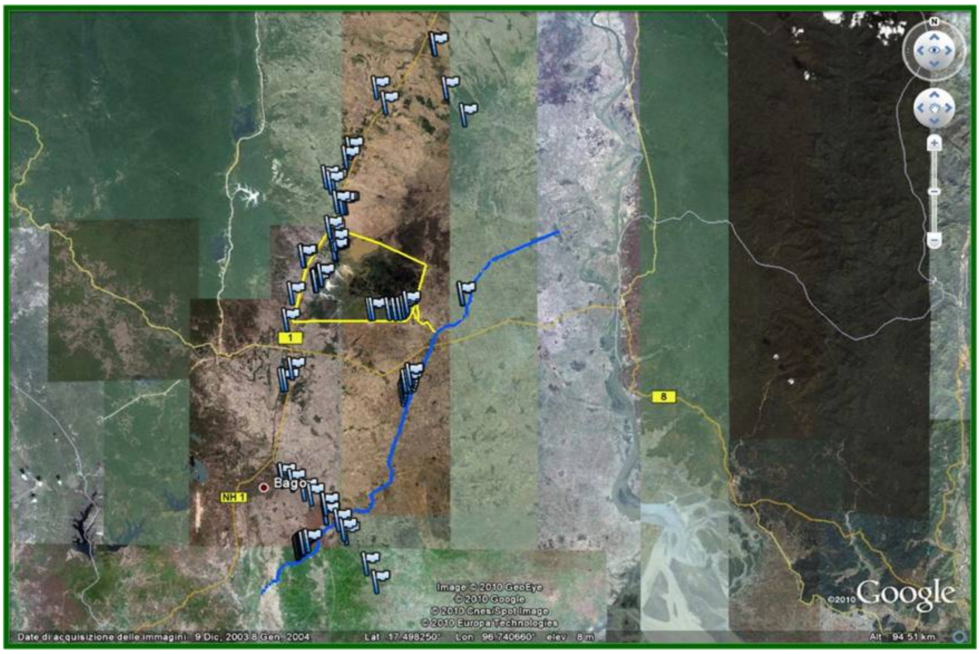
6.2.5. Geographic distribution of farms

The farms sampled are distributed in the above described strata as reported in Table 3 (see next page).

Table 3: Distribution of farms investigated per strata

Stratum	N° of farms	% of the investated farms
Bank	9	11.25
Canal	34	42.50
Lake	11	13.75
Roadside	26	32.50
TOTALE	80	100

Figure 8: Geographic location of the sampled farms (GPS coordinates).



7. RESULTS OF THE EPIDEMIOLOGICAL STUDY

This chapter reports results of the epidemiological analysis. Results come from laboratory testing of 4,260 duck serum samples, 5,290 cloacal swabs and 1,060 tracheal swabs which were collected during 142 farm sampling visit completed across 80 farms over 5 study surveys.

7.1 Results of serum positivity in a nutshell

16 duck farms out of the 80 investigated left the study. None of them were affected by AI or any other infection before leaving the study. 15 of these farms left the study between January and March, between the last and two sampling rounds.

Positivity results are a follow:

- 61 farms were positive on a serological basis
- 644 sera out of the 1830 tested were positive (35%.)
- The average number of positive serum sera out of the 30 specimen samples collected in each flock amounted to 11 (37%).

By the end of the study farms had turned positive in the percentages shown in Table 4 (see next page).

Table 4: Positive and negative farms per stratum (farms which left are disaggregated and accounted for separately)

Stratum	Neg (%)	Pos (%	Out (%)	
Bank	1 (11,1)	6 (66,7)	2 (22,2)	9 (100)
Canal	1 (2,9)	27 (79,4)	6 (17,6)	34 (100)
Lake	0 (0)	9 (81,8)	2 (18,2)	11 (100)
Roadside	1 (3,8)	19 (73,0)	6 (23,0)	26 (100)
TOTAL	3 (3,7)	61 (76,2)	16 (20,0)	80 (100)

If the farms which left the study are not accounted for, positive farms in all strata add up to approximately 100% (Table 5).

Table 5: Positive and negative farms per stratum (farms which left the study are NOT disaggregated).

Stratum	Neg (%)	Pos (%)	Total (%)
Bank	1 (14)	6 (86)	7 (100)
Canal	1 (4)	27 (96)	28 (100)
Lake	0 (0)	9 (100)	9 (100)
Roadside	1 (5)	19 (95)	20 (100)
TOTAL	3 (5)	61 (95)	64 (100)

7.2 General description of serological results in duck flocks

The number of ducks which seroconverted from the pool of birds sampled at each farm is different across strata as reported in Table 6.

Table 6: Mean number of seropositive ducks in the sampled pool of specimens of each positive farm.

Stratum	No. pos. farms	Mean number of positive sera in the pool of 30 specimens
Bank	6	4
Canal	27	12
Lake	9	12
Roadside	19	10

Given the reduced number of farms located in the bank and lake areas the epidemiological analysis focuses only on the roadside and canal areas. The analysis of the distribution of positive samples uses percentage values. When the median instead of the mean of positive samples is used no significant statistical distributions between the area canal and roadside areas are observed (Wilcoxon rank test, p-value obs= 0.4855).

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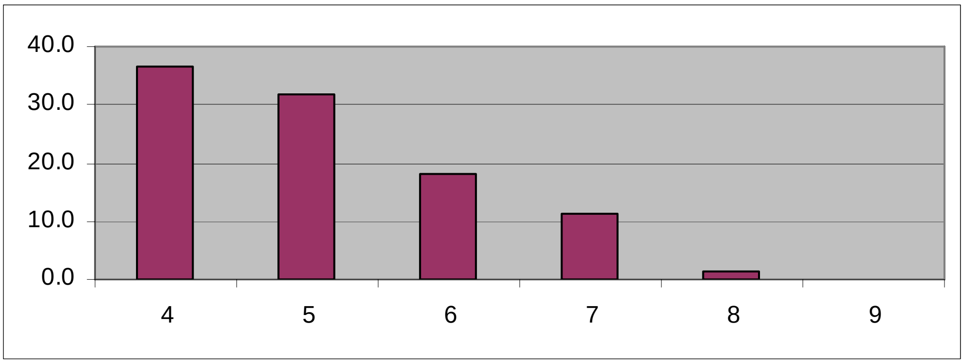
The median of HI Ab titres is log 5 in all the strata. 50% of positive sera have a titre ranging between log 5 and log 6. Only 2% of positive sera show a titre higher than log 8. The frequency distribution of the HI is shown in Table 7 and diagrammed in Graph 1.

Table 7: Frequency distribution of the serological titres recorded during all the study.

Log	N of positive	%
4	236	36.5
5	206	31.99
6	117	18.17
7	74	11.49
8	10	1.55
9	1	0.16
TOTAL	644	

Graph 1: Frequency distribution of the serological titres positive for HI (logs are reported in x axis).



No differences among areas appear when the HI AB titres are considered for analysis. This can be seen in Table 8 which details the mean and the percentile distribution of the log titre of the HI test.

Table 8: Distribution of means and percentiles of the serological titres tested with HI.

Stratum	N	Mean	P50	P25	P75	max
Bank	21	5,2	5	4	6	7
Canal	313	5,1	5	4	6	9
Lake	112	5,2	5	4	6	8
Roadside	198	5,1	5	4	6	8
TOTAL	644	5,1	5	4	6	9

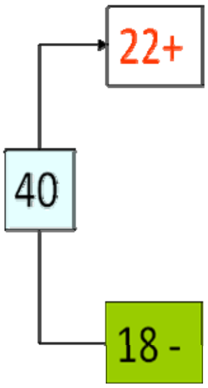
The median is 5 in all four areas and 50% of samples have a titre level variable between log 4 and 6. The analysis of the frequency of distribution of titres (Table 7) shows that around 70% of positive samples have HI log titres of 4 and 5 and that 30% have HI log titres of 6 – 8 and around 2% have HI log titres of 8 and 9.

7.3 Results of the samplings rounds

7.3.1 First sampling (September 2009)

Samples from 22 out of 40 duck farms showed Ab against the H5 AIV subtype while the remaining 18 farms showed negative samples (Diagram 2), a 55% prevalence rate. Virus isolation performed by the inoculation of embryonated eggs was negative in all samples.

Diagram 2: Farms positive at first sampling for H5 AIV subtype



7.3.2. Second sampling (October 2009)

4 out of the 18 negative farms seroconverted at this sampling (Diagram 3) showing newly elicited Ab against the H5 AIV subtype. The incidence rate was 22%. Seroconversions occurred in the strata canal, lake and roadside strata (Graph 2). Virus isolation performed by the inoculation of embryonated eggs was negative in all samples

Graph 2: Seroconversion in strata at second sampling.

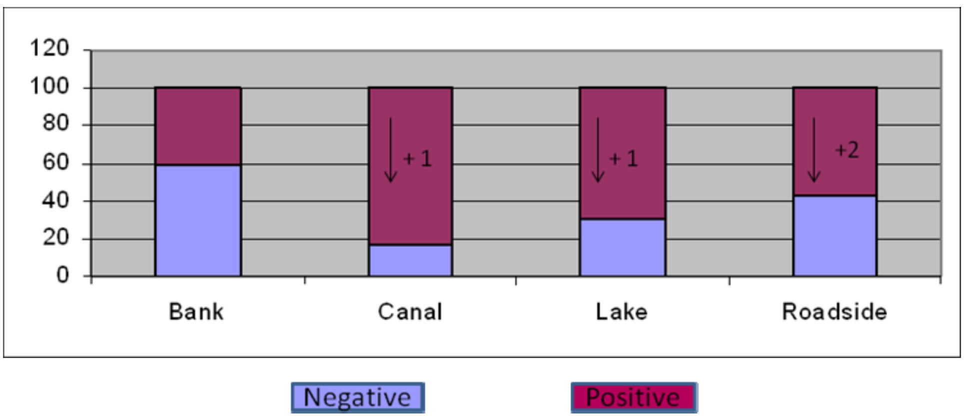
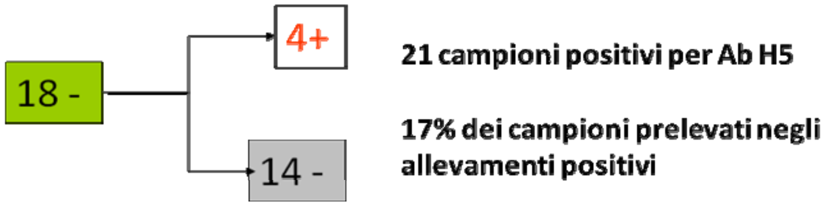


Diagram 3: New farms positive to H5 AIV subtype at second sampling



7.3.3 Third sampling (November 2009)

2 out of the 13 negative and available farms seroconverted[2] thus showing and incidence rate of **15%.** The new cases were both located in the Lake stratum (Graph3).

22 out of the 40 new farms introduced with the second cross sectional study (Figure 1) tested positive (Diagram 4) giving a prevalence rate of 55%. This rate is the same as the prevalence rate of the first cross sectional sampling carried out in September. Farms found positive at the second cross sectional study were distributed between strata as outlined in Graph 4.

[2] Accounting of farms sampled is facilitated by consultation of Figure 1

Graph 3: Seroconversion in strata at third sampling.

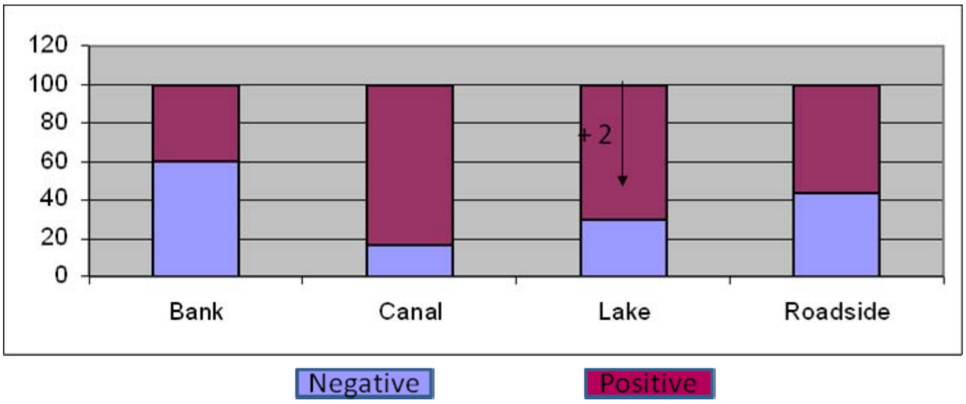
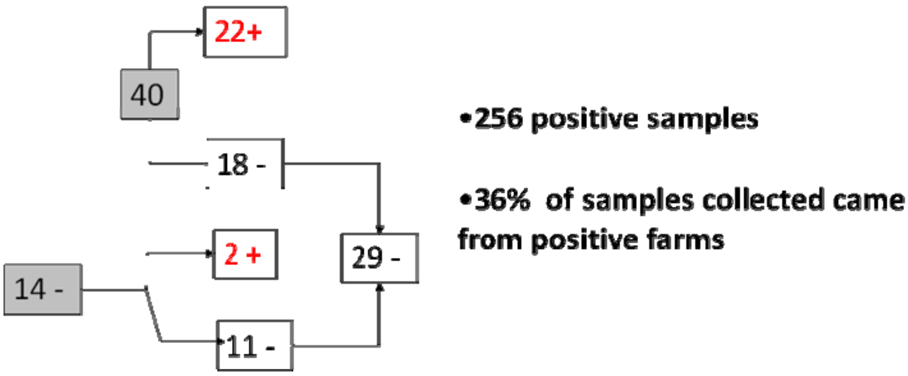
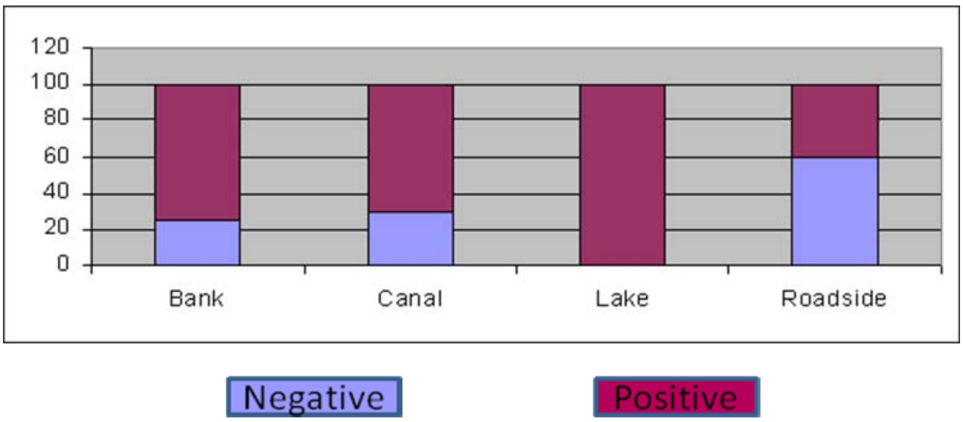


Diagram 4: New farms positive for H5 AIV subtype at the third sampling (Includes second cross sectional sampling)



Graph 4: Serum positive farms at second cross sectional study by strata.



7.3.4 Fourth sampling (January 2010)

5 out of 21 farms seroconverted (Diagram 5). Taking into consideration that 8 farms left the study (Figure 1), the incidence rate was 24%.

Graph 5: Serum positive farms per strata at fourth sampling.

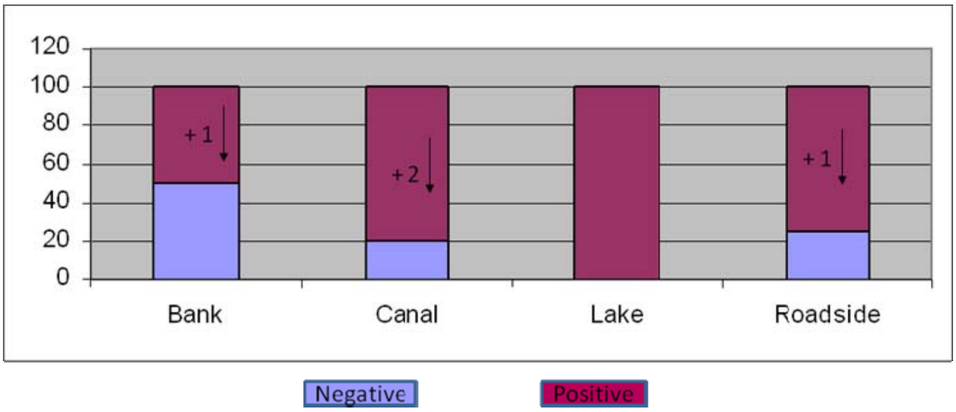
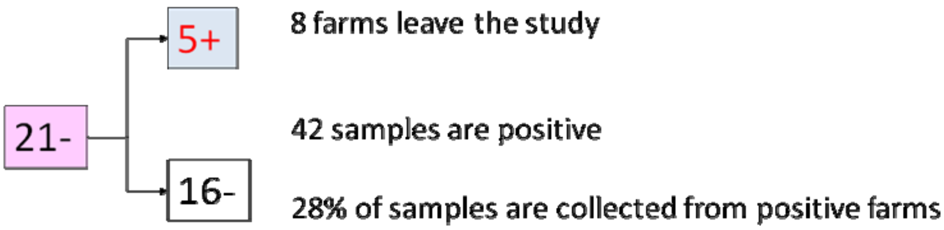


Diagram 5: New farms positive for H5 AIV subtype at the fourth sampling



7.3.5. Fifth sampling (March 2010)

Some farms left the study and were not available for this sampling (see the outline in Figure 1). 7 out of the 16 farms identified as negative during the fourth sampling tested HI serologically positive for H5 AIV. Diagram 6 lays out the positive farms showing only those available for sampling. The lake stratum is not represented anymore while the canal and roadside strata still have four farms each. Three out of these four farms per stratum are positive (Graph 6). The last farm in the bank area is negative. In conclusion 6 out of the 9 farms that remained available for sampling seroconverted, which is an incidence rate of 67%.

Graph 6: Serum positive farms at fifth sampling per strata (the lake stratum is not represented anymore).

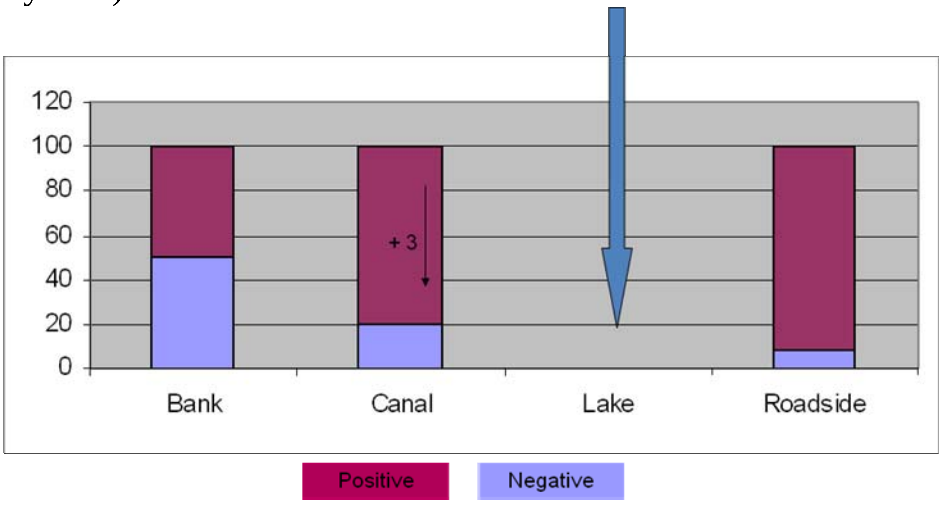
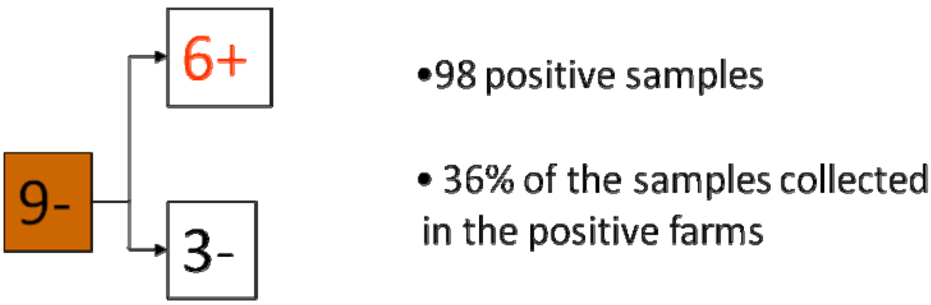


Diagram 6: New farms positive for H5 AIV subtype at the fifth sampling



It is worth noting that the distribution of the H5 subtype HI serological titres shown by this sampling round (Table 9) is more variable than that of the other samplings (for comparison with the log titre distribution of the entire study see Table 7).

Table 9: Frequency distribution of serological titres from the fifth sampling.

Log	N of positive sera	%
4	19	19.39
5	24	24.49
6	23	23.47
7	23	23.47
8	9	9.18
TOTAL	98	100

7.4 Results of the additional attempt to identify the virus

Three farms were investigated all located in the canal area. The AIV was not isolated from any of them. RT-PCR tested positive for gene M but no genes of the subtypes H5, H7 and H9 were identified. Results are tabled below and presented together with their serological history.

Table 10: Result of the special attempt to isolate virus from farm DTC003

Population	600 ducks 9 month-old 160 ducks 4 month-old
Serological status in January 2010	30/30 negative sera for Ab against the H5 subtype
Serological status in Mach 2010	12/50 positive sera for H5 AIV subtype (log 4 and 5) tested with locally produced Antigen (Ag) and confirmed at IZSVe using standard Ag
Clinical symptoms	Depression, respiratory signs, reduced growth in 4 month old birds
Results of virus isolation	470/600 ducks of 9 months → neg 160/160 ducks of 4 months → neg
PCR Results for gene M	Cloacal swabs positive between 23 and 31 thermal cycles. Tracheal swabs negative.
PCR results for the research of gene H5 (by means of RT and standard), H7, H9	Negative

Table 11: Result of the special attempt to isolate virus from farm DTC002

Population	300 ducks
Serological status in January 2010	30/30 negative sera for Ab against the H5 subtype
Serological status in Mach 2010	29/30 positive sera for H5 AIV subtype
Clinical symptoms	none
Results of virus isolation	286 tracheal and cloacal swabs all negative
PCR Results for gene M	Cloacal swabs positive between 23 and 29 thermal cycles. Tracheal swabs negative.
PCR results for the research of gene H5 (by means of RT and standard), H7, H9	negative

Table 12: Result of the special attempt to isolate virus from farm DTC001

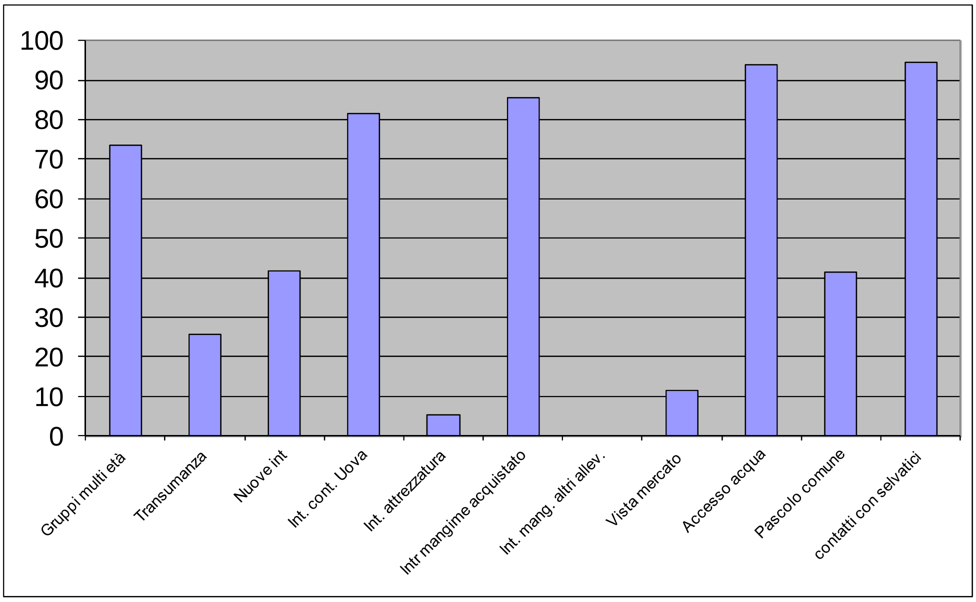
Population	500 ducks
Serological status in January 2010	30/30 negative sera for Ab against the H5 subtype
Serological status in Mach 2010	25/30 sera positive for Ab against the AIV H5 (log 4 -8)
Clinical symptoms	none
Results of virus isolation	100 cloacal and tracheal swabs negatives
PCR Results for gene M	1 out of 20 pools of Cloacal Swabs positive at 27 thermal cycles. Tracheal Swabs negative
PCR results for the research of gene H5 (by means of RT and standard), H7, H9	negative

7.5 Results of the risk factors investigated and recorded

Risk factors identified during AEA (see paragraph 4.2.3) and investigated by means of questionnaires (see paragraph 4.3.3) are reported in

Graph **7**. Logistic regression to identify possible risk factors determining AI seroconversion was actually attempted but was analytically impossible due to lack of consistent recordings throughout the study as well as the progressively smaller numbers of farms as the end of the study came closer.

Graph 7: Frequency distribution of AI introduction risk factors



7.6 Survival analysis

Survival analysis was carried out on 29 out of the 80 duck farms investigated. The smaller number analyzed out of all the farms which entered the study was due to the exclusion of 44 farms at the initial cross sectional surveys and the exit of another 7 farms immediately after the first sampling.

Furthermore it is necessary to mention that the number of duck farms at the end of the study was sensitively reduced in comparison to the initial number analyzed. This problem reduced the statistical soundness of the results. It is also remarkable that only one out of all the observed duck farms did not show the occurrence of any "event" at the end of the observation.

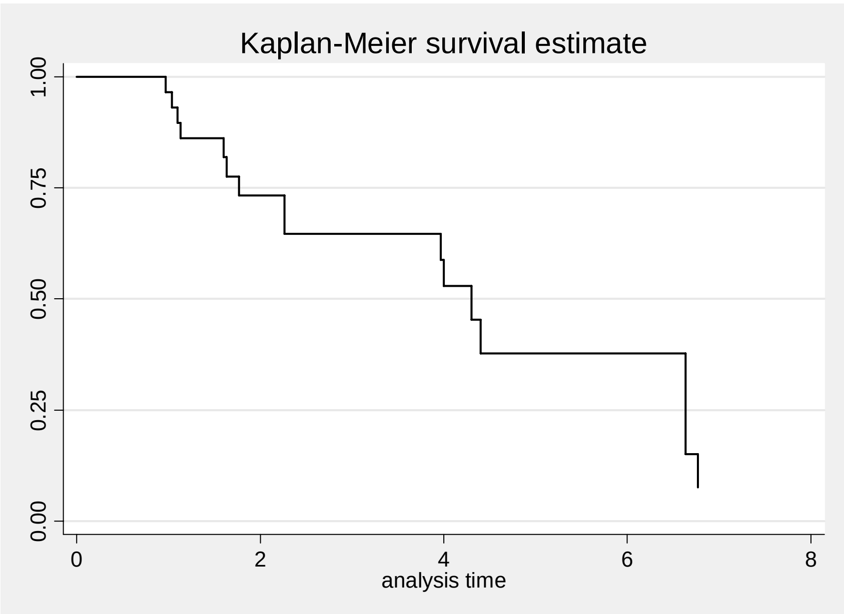
Results of survival analysis are reported in Table 13.

Table 13: Result of Survival Analysis

Time	Total	Fail	Lost	Survivor Function	Error	[95% Conf.	Int.]
.97	29	1	0	0.97	0.03	0.78	0.99
1.03	28	1	0	0.93	0.05	0.75	0.98
1.1	27	1	0	0.90	0.06	0.71	0.96
1.13	26	1	1	0.86	0.06	0.67	0.94
1.4	24	0	4	0.86	0.06	0.67	0.95
1.6	20	1	0	0.82	0.07	0.62	0.92
1.63	19	1	0	0.78	0.08	0.56	0.89
1.76	18	1	0	0.73	0.09	0.52	0.86
2.27	17	2	0	0.65	0.10	0.43	0.80
2.37	15	0	1	0.65	0.1	0.43	0.80
3.87	14	0	3	0.65	0.1	0.43	0.80
3.97	11	1	0	0.59	0.10	0.36	0.76
4	10	1	0	0.53	0.11	0.30	0.71
4.23	9	0	2	0.53	0.11	0.30	0.71
4.3	7	1	0	0.45	0.12	0.22	0.65
4.4	6	1	0	0.38	0.12	0.16	0.60
6.63	5	3	0	0.15	0.10	0.03	0.37
6.77	2	1	1	0.08	0.07	0.00	0.28

Interpretation is done by considering columns “time” and “survivor function”. The first reports the time of follow up expressed in months. The second reports the probability of infection. One month from the first sampling, the probability of non infection is 93% while after 3 months it is about 65%. At around more than 4 months the probability of non infection is dramatically reduced to less than 38% and it finally reaches almost zero by the end of the study. It is worth mentioning that the number of farms was substantially reduced at the end of the study, thus impairing the soundness of the analysis. The graphical representation of the analysis is the Kaplan-Meier curve represented in Graph 8.

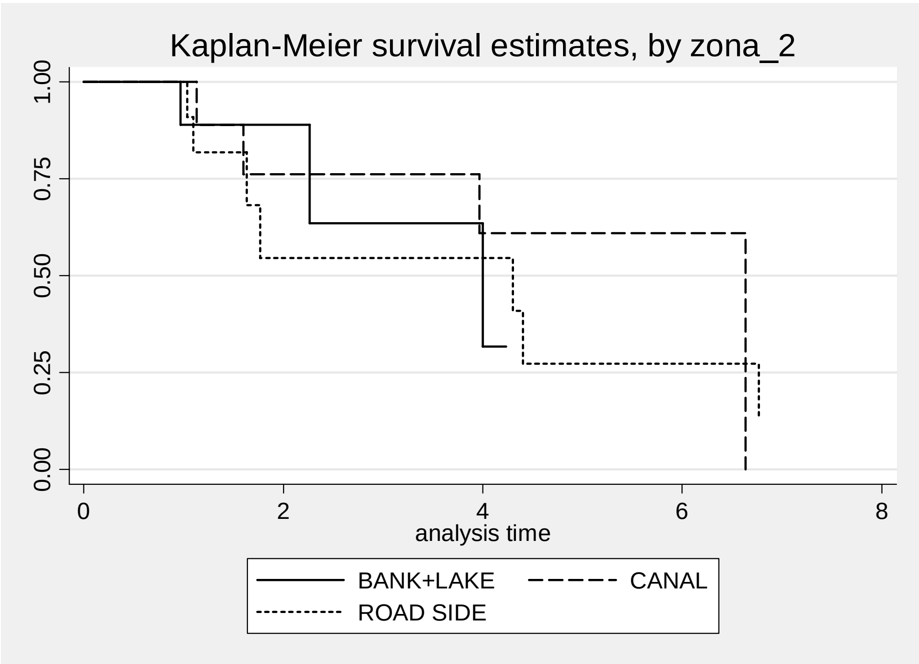
Graph 8: Kaplan – Meyer curve of the event seroconversion analyzed throughout the entire study (time is expressed in months).



The median survival time (the mean time of non seroconversion) is 4.23 months.

Specific curves have been drawn in order to enable the comparison among different areas or agro-ecosystems (or strata). Due to the scarcity of farms representing the bank and lake areas these strata were merged into one. This was possible because they were deemed the most similar from an epidemiological and agro-ecological perspective. The comparison is outlined in Graph 9.

Graph 9: Comparison of the Kaplan Meyer curves representing different strata.



The 3 curves do not show statistical significant differences (p-value long-rank test: 0.9289, p-value Wilcoxon test: 0.8175).

8. DISCUSSION AND CONCLUSIONS

8.1. Environment, duck husbandry and the agro-ecosystem.

The Moeyingyi wetland represents a very complex agro-ecosystem. Its swampy area was transformed into a man governed watershed / irrigation system whose control is given up to nature during the apex of the monsoon season. The Moeyingyi swamp has been actually transformed into a lake that has an extension and shape variable according to the season. The man made intervention on the environment carried out by the British colonizers by digging the Bago-Sittaung canal (stratum “canal”) was completed by the construction of the south lake earth-bund which has several water gates (this area corresponds to the stratum “bank” of the study). The first of these two infrastructures created a fluvial waterway, the second created a water irrigation system with the double purpose of: (1) channeling water for rice fields’ irrigation allowing two crops per year, (2) create a lake for the establishment of a wild bird sanctuary.

These interventions have increased the human settlements in the are and made residents’ livelihood more sustainable

The earning originating from the touristic exploitation of the wild bird sanctuary do not directly benefit the local population. At time of investigation the touristic potential of the wild bird sanctuary appeared underexploited. Land reclamation made possible a more profitable rice cultivation and the integrated duck raising but, unfortunately created the conditions for the introduction and circulation of the AIV. The presence of one of the main routes connecting Lower with Higher Myanmar (characterizing the area called "road side") increased the possibilities for the infection spread along with duck sale. Indeed the area is the main duck hatchery of Myanmar producing 60% of the national duck flock.

4000 families engage in duck raising and a large number is indirectly involved in the sector. At present an attempt to manage the size of the duck population seems indeed very difficult because there are no alternatives for a decent livelihood and rice production alone is not totally sufficient for sustaining dwellers' living. Many rice farmers are also duck farmers and this complementary activity is necessary. Moreover the income differentiation is key to distributing enterprise risks. The area has probably a promising potential for the development of eco-tourism. This doctoral research suggests that long term strategies are necessary for pursuing changes of the local economy towards a system which relies less on traditional low biosecurity duck farms and which entails the reduction of duck farms and their better sanitary control. Provided that the preparatory phases investigated widely on environmental and social aspects and given that socio-economic collateral studies were implemented, suggestions are also given to approach holistically farmers' problems when specific AI control impact is sought. A sectoral approach seems proper and realistic.

8.2 Discussion of the epidemiological study results

The most noteworthy results are the high seroprevalence for the H5 AIV subtype and the high incidence of the infection in negative farms which is balanced by negative virological testing. For this reason, it was not possible to ascertain whether the circulating virus was a high or low pathogenicity type. The hypothesis about the origin of the infection as spread from a quail farm outbreak occurred two years before the start of the present study in Bago was not verified.

In this study the identification of the virus pathogenicity type was never supported by the clinical observation on the chicken and quail susceptible populations which were negligible. The circulation of HPAIV can be only suspected from the very high serological titres of the infected ducks which were also confirmed by the OIE/FAO AI and Newcastle Disease Reference laboratory at IZSVe. Yet, such a explanation is only useful for formulating hypotheses to be tested. The very high Ab titres against the H5 subtype were collected from duck showing clinical symptoms compatible with HPAI. For this reason three flocks were investigated *ad hoc* and showed again negative results to virological testing done by means of RT-PCR and Virus Isolation. Their high log could also be generated by the natural booster of re-circulating viruses.

If the virus presence can be indirectly argued, virus isolation is still necessary for the local veterinary services to convince the government to implement expensive and unpopular control measures. Actually ,according to the OIE, the circulation of a H5 notifiable AIV subtype also referred to as Notifiable Avian Influenza Virus (NAIV) ascertained serologically makes the notification and reporting of the infection compulsory also without virus isolation and irrespective of the H5 subtype pathogenicity. Endemic developing and poor countries will hardly implement control measures based on barely symptomatic duck flocks. The implemented epidemiological study and the surveillance scheme tested represents a valuable suggestion. It allows allocating finances to produce sufficient information to recognize the presence of HPAIV or LPAIV. Sensitive veterinary service can take advantage and try managing territorial strategies for addressing the issue.

The above statement derives from the tested approach based on the critical interpretation of serological data in order to select actively shedding flocks. As described, duck farms of very recent infection have been sampled with a protocol envisaging the collection of a very large proportion of the flock. Notwithstanding, laboratory tests did not help to get to undisputable conclusions because they were virologically negative for NAIV. The reason for samples to be negative in face of serological positivity for H5 lies in the intermittent and short viral shedding from infected ducks (Beato et al, 2007). The virological samples tested RT-PCR positive for Type A Influenza viruses (detection of gene M) but were always negative for Highly Pathogenic subtypes. It is necessary to take into account that the non ideal conservation of sample is a further possible cause of unsuccessful viral ascertainment because samples remained in Myanmar longer than expected for bureaucratic reasons in critical refrigeration conditions. Results of the serological analyses have anyway enabled performing a survival analysis which has identified and quantified the time of infection spread among flocks along an entire production cycle (September 2009 to March 2010).

It is anyway recognized that virus isolation would have allowed to take decision easier and also to follow up with the implementation of post outbreak epidemiological investigations at the country level. In addition, epidemiological molecular investigation would have helped to investigate the hypothesis that the Moeyingyi area generated some of the AIV circulated in Myanmar. This hypothesis is formulated taking in consideration that the commercial network for duck sale has the reproduction center in the Moueyingyi area.

8.2.1. Survival analysis

From the survival analysis it is possible to make the following considerations:

- In October, after 2 months from first sampling, farms located in the road side area show a lower probability to remain uninfected (circa 54%) in comparison to farms located in the canal and bank areas (both showing a probability to remain uninfected of 75%). In other words, roadside farms are more probably infected.

- In December, after 3 months from first sampling, farms located in the canal area are still not infected (76% probability) while those in the area bank + lake show a probability of seroconversion close to that of the “road side” farms.

- In January, after 4 months from first sampling, probability of being uninfected for farms located in the bank + lake area accounts for around 30%. While in March (six months after first sampling) in the canal and road side area there are still farms which have not yet seroconverted.

The survival analysis suggests that starting a new production cycle with new control measures in place is imperative for controlling AI because ducklings are initially negative but get infected very quickly. The control measures to be considered are depopulation, increased biosecurity and vaccination. Depopulation is for socio-economic and ethical reasons impossible unless alternative livelihood is provided which requires long term rural development strategies. Biosecurity in a wetland densely populated by wild birds is not effective alone. Vaccination might be an option if it could be planned and executed under strict control. Anyway, given the difficulties for virus isolation and provided that serological results remain the more reliable indicator of infection, eliciting a vaccine generated immunity on the population would lead to losing the only one indicator of infection which would end up with losing the only possibility of infection monitoring. DIVA (Differentiate Infected by Vaccinated Animals) systems are a theoretical solution for developing countries but are not used in SE Asian countries. As supported by the survival analysis results, any control measure should be prioritized in the roadside area.

8.3 Synoptic conclusions

- High serum positivity for H5AIV subtype was found in all the areas and in all the flocks investigated with the exception of one case,

- Rapid seroconversion of negative farms as shown by the survival analysis was observed,

- High circulation of H5 AIV subtype could be inferred,

- No clinical symptoms with the exception of farm DTC003, and with minor extent farms DC002 and DC001, were observed,
- Virus isolation even when searched with timely and strategic methods for targeting shedding was never obtained by means of RT-PCR and virus isolation. This is explained with:
    - The short period of shedding (Beato et al, 2007)
    - Low viral titres (Beato et al, 2007)
    - Bad conservation of samples probably occurred during the study
- Serology is a useful tool for a sound surveillance policy in endemic developing countries,
- Myanmar is a challenging case to set up control measures suitable for other developing countries,
- This study is the first attempt to provide information and epidemiological scientific knowledge on AI from Myanmar

9. RECOMMENDATIONS FOR THE MYANMAR VETERINARY SERVICES

Based on the present study it is suggested to continue attempting virus isolation through the early identification of seroconverted flocks which must be immediately sampled and to adopt serology for routine surveillance.

It is recommended to approach farmers with participatory methods and to take into account their livelihood because, given the absence of apparent clinical symptoms and associated economic losses, AI is, in duck farmers' conception, one of their minor problems.

It is suggested to compare the results produced by the present surveillance scheme with those obtained after control measures are implemented.

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